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Contents

M.K. Kar, , M.V.R. Rao, T.N. Sahoo , G.J.N. Rao and A.T. Roy
ASSESSMENT OF GENETIC DIVERSITY IN SHORT GRAINED AROMATIC RICE USING CONVENTIONAL AND MOLECULAR APPROACHES . 1-18

Humeraha Nazneen and S. Karanakar Reddy
INDUCTION OF CALLUS FROM DIFFERENT EXPLANTS IN STEVIA REBAUDIANA. 19-22

Ademiluyi, Benson Oluwafemi and Arowosegbe S.
EFFECT OF PRE-PLANT HERBICIDES ON WEED CONTROL AND PERFORMANCE OF OKRA *ABELMOSCHUS ESCULENTUS* IN A SOUTH WESTERN NIGERIAN LOCATION. 23-27

A. Premila Devi
AEROMYCOFLORA OF A COLLEGE LIBRARY IN IMPHAL. 29-36

Ayanbamiji, T. A. and Ogundipe, O. T.
EFFECT OF CEMENT DUST ON THE MORPHOLOGY AND LEVELS OF SOME METALS IN *ELEUSINE INDICA* L. GUERTN AND *SIDA ACUTA* BURN F. 37-45

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Contents

Laxmi Manandhar and Belai Meeta Singh Suwal
CONTRIBUTION TO A CHROMOSOME ATLAS OF THE NEPALESE FLORA I. 37-51

B.C.Aharya , H.N.Subudhi and S.P.Panda
CHECK LIST OF ECONOMIC PLANTS OF ROURKELA AND ADJOINING REGIONS (SUNDARGARH DISTRICT)-ODISHA. 53-57

A.Premila Devi
AEROMYCOFLORA OF A CABBAGE FIELD IN IMPHAL. 59- 62

J. Kayode and M. J. Ayeni
ALLELOPATHIC POTENTIALS OF AQUEOUS EXTRACTS FROM MAIZE (*ZEA MAYS*) ROOTS AND PIGEON PEA (*CAJANUS CAJAN* L.) LEAVES ON THE GROWTH AND GERMINATION OF SOYBEANS (*GLYCINE MAX.* L. MERRIL.). 63-68

R. Singh and M.U. Charaya
EFFECT OF UREA AND SINGLE SUPER PHOSPHATE ON *IN VITRO* DECOMPOSITION OF WHEAT CROP RESIDUES BY *TRICHODERMA LIGNORUM.* 69-73

Y. P. Patil, S. V. Borle and C. M. Bharambe
IMPACT OF FLYASH WATER ON BIOCHEMICAL CONTENTS OF *ARACHIS HYPOGEA L.* 75-77

B. Muthukumar[1] and M. N. Abubacker
STUDIES ON *IN VITRO* ANTIMICROBIAL ACTIVITIES OF LEAF EXTRACTS OF *DATURA METEL* LINN. 79 – 82

G.Ramanjaneyulu , P.Seshapani , B.V.Ramana Naidu , D.Jayasimha Rayalu, Prabhakar Raju, C., and J. Pramoda Kumari
GENOME WIDE ANALYSIS AND IDENTIFICATION OF GENES RELATED TO CYCLIC NUCLEOTIDE GATED CHANNELS (CNGC) IN *ORYZA SATIVA.* 83 - 91

H.N. Subudhi , J.Meher and K.S.Behera
PERFORMANCE OF SOME PROMISING RICE VARIETIES IN LATE PLANTED CONDITIONS. 93-96

Laxmi Manandhar, Buddha Laxmi Vaidya and Belai Meeta Singh Suwal
CONTRIBUTION TO THE CHROMOSOME ATLAS OF THE NEPALESE FLORA II. 97-112

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ASSESSMENT OF GENETIC DIVERSITY IN SHORT GRAINED AROMATIC RICE USING CONVENTIONAL AND MOLECULAR APPROACHES

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ABSTRACT

Agro-physico chemical traits and RAPD markers were used to survey genetic diversity in 47 rice genotypes which included 38 non-basmati local aromatic rice varieties, five basmati varieties and four non-aromatic high yielding slender grain varieties. Seven agronomical characters and twelve physico-chemical characters were studied and field as well as laboratory data were subjected to multivariate analysis as proposed by Mahalanobis's D^2-statistics and Tochers method of clustering. Molecular characterization of the genoypes was done using thirty decamer primers from Bangalore Genei Pvt. Ltd. The 47 genotypes were classified into six clusters based on D^2 analysis. All the local scented varieties were grouped in to the cluster I excepting Dubraj and Mahulkuchi. In the cluster II, three aromatic long grained basmati type varieties and three non-aromatic long grained varieties were present, while in cluster III, two medium grained varieties, one being aromatic (Dubraj) and another non-aromatic (Sambamahsuri) were clubbed together. Two basmati type aromatic varieties Pusa Basmati 1 and Kernal Local related by ancestry formed two distinct monogenotypic clusters. Thus D^2 analysis could not give a clear cut classification of aromatic and non-aromatic rice varieties. Besides that it could not throw some light on ancestry also. On the other hand, from the dendrogram constructed based on RAPD data, at 75% similarity coefficient level, all the aromatic varieties were clubbed in to a single cluster excepting two local varieties Dubraj and Sujata. The non-aromatic varieties also formed a distinct cluster. At a higher similarity level, the local aromatic rice and basmati types formed two distinct sub-clusters. Thus, from the present study it can be inferred that RAPD analysis can be used as a reliable method for classification of rice varieties into local aromatic, basmati type and non-aromatic types.

Key words : Aromatic rice, Mahalanobis's D^2-statistics, RAPD.

INTRODUCTION

Aromatic rices are a gift of nature to mankind. These constitute a small but special group of rices and have occupied a prime position in Indian Society. Earlier, a large number of aromatic rices were collectively known as Basmati. Over the years, the definition of Basmati has changed and it is increasingly restricted to long slender grain type with moderate to strong aroma (Mahindru, 1995). Accordingly, the small and medium grain aromatic rices are being regarded as a separate class of non-Basmati aromatic rices. India is a leading country in the world for export of aromatic rices, particularly Basmati rices. So, a lot of emphasis has already been given for the genetic up gradation of such Basmati types. But the non-Basmati short grained aromatic rices are a neglected lot. A lot of these rices have already been lost as an aftermath of green revolution, where major emphasis was on yield rather than quality. These land races are highly adaptable to local environments with pleasant thermo-stable aroma and resistance to major diseases and pests. These traditional varieties are tall, photosensitive with moderate to very strong aroma, good kernel elongation after cooking, but are medium to long duration and phenomenally low yielders. Thus, further progress in the improvement of these aromatic rices needs immediate conservation of genetic diversity, their evaluation and effective utilization.

Characterization and analysis of available genetic diversity constitute an indispensable prelude to planning and development of breeding strategies. For genetic up gradation of any crop, assessment of available genetic diversity in the germplasm would be a definite step forward. In the present study, an attempt has been made to study the genetic diversity of the short-grained aromatic rice varieties both at molecular level using RAPD analysis and morpho- physico-chemical level using D^2 analysis.

MATERIALS AND METHODS

Plant materials

A total of 47 rice genotypes were used for the present investigation. Of these, 38 were short grained aromatic varieties, 5 were basmati type long grained aromatic varieties and 4 were non-aromatic high yielding varieties (Table 1).

D^2 Analysis

The experiment was conducted at the Rice Research Station, Bhubaneswar. Seedlings were nursery raised and transplanted at thirty-day age in the experimental plots. The field lay out was randomized complete block design with two replications. Each plot constituted five rows of 24 hills per row with row-hill spacing of 20 ×15cm. Fertilizers were applied at the rate of 60 kg N, 30 kg P_2O_5 and 30 kg K_2O per hectare in a manner of standard practice. Plant protection measures taken were need based. Observations were recorded on seven morphological characters like days to 50% flowering, plant height, panicle number, panicle length, grain number per panicle, grain weight and plot yield. Days to 50% flowering and plot yield were studied on plot basis. Observations on all other traits were recorded on ten competitive random plants selected from the middle three rows in each replication. The varieties were also evaluated for twelve physico-chemical characters like hulling percentage, milling percentage, head rice recovery percentage, kernel length, kernel breadth, L/B ratio, kernel length after cooking, elongation ratio, volume expansion, water uptake, alkali spreading value and amylose content in the Biochemistry Laboratory of Central Rice Research Institute (CRRI), Cuttack. The data were analyzed for Mahalanobis' D^2 statistic to measure the genetic divergence among the test entries and their grouping into different clusters was done by Tocher's method (Rao, 1952).

Table 1 : Important features of the varieties included in Experiment I and II

Sl.	Varieties	Origin	Aroma	Grain character
1.	Badsabhog	Orissa	Present	Short bold
2.	Baranamgomati	Orissa	Present	Short bold
3.	Barikunja	Orissa	Present	Short bold
4.	Basanasapuri	Orissa	Present	Short bold
5.	Basuabhoga	Orissa	Present	Short bold
6.	Chhatianaki	Orissa	Present	Short bold
7.	Dubraj	Orissa	Present	Medium
8.	Dhoiabankoi	Orissa	Present	Short bold
9.	Dubrajsena	Orissa	Present	Short bold
10.	Jala	Orissa	Present	Short bold
11.	Jalaka	Orissa	Present	Short bold
12.	Jhillipanjari	Orissa	Present	Short bold
13.	Jhingisiali	Orissa	Present	Short bold
14.	Kalajira	Orissa	Present	Short bold
15	Kalajiri	Orissa	Present	Short bold
16.	Karpurakali	Orissa	Present	Short bold
17.	Kalikati	Orissa	Present	Short bold
18.	Khosakani	Orissa	Present	Short bold
19.	Mahulakuchi	Orissa	Present	Short bold
20.	Kalakrushna	Orissa	Present	Short bold
21.	Lektimachi	Orissa	Present	Short bold
22.	Manasi	Orissa	Present	Short bold
23.	Leelabati	Orissa	Present	Short bold
24.	Nalidhan	Orissa	Present	Short bold
25.	Parijatak	Orissa	Present	Short bold
26.	Ratnasundari	Orissa	Present	Short bold
27.	Sirimula	Orissa	Present	Short bold
28.	Sujata	Orissa	Present	Short slender
29.	Thakurabhoga	Orissa	Present	Short bold
30.	Thakurasuna	Orissa	Present	Short bold
31.	Parbatajira	Orissa	Present	Short bold
32.	Tulasiphulla	Orissa	Present	Short bold
33.	Tulasibasa	Orissa	Present	Short bold
34.	Durgabhoga	Orissa	Present	Medium

35.	Pimpudibasa	Orissa	Present	Short bold
36.	Nanu	Orissa	Present	Short bold
37.	Ganjam Local 1	Orissa	Present	Short bold
38.	Ganjam Local 2	Orissa	Present	Short bold
39.	Pusa Basmati 1	IARI, New Delhi	Present	Long slender
40.	Taroari Basmati	North-West India	Present	Long slender
41.	Sanwal Basmati	North-West India	Present	Long slender
42.	Karnal Local	North-West India	Present	Long slender
43.	Basmati 370	North-West India	Present	Long slender
44.	Sambamahsuri	Bapatla, AP	Absent	Medium
45.	IR 64	IRRI	Absent	Long slender
46.	Lalat	OUAT,Bhubaneswar	Absent	Long slender
47.	PR 106	PAU, Ludhiana	Absent	slender

RAPD Analysis

Plant DNA extraction

Total DNA was isolated from green leaves of the 47 rice genotypes by the cetyltrimethyl ammonium bromide (CTAB) method of Murray and Thompson (1980). Twenty days old seedlings were bulked and two grams of tissue was ground to a very fine powder in liquid nitrogen. Approximately 350 mg of the powder was taken in each of the six microfuge tubes and equal volume (w/v) of hot 65^0c 2x CTAB buffer (2% CTAB; 1.4M Nacl; 20mM EDTA,pH 8.0; 100mM Tris, pH 8.0 and 2% mercaptoethanol) was added . The content of each tube was mixed thoroughly by inversion and equal volume (700µl v/v) of ice cold chloroform: isoamyl alcohol (24:1) was added. After mixing thoroughly by inversion to form an emulsion the tubes were centrifuged at 15000rpm. Using a cut yellow tip, the top (aqueous) phase was transferred to a fresh tube .The volume was measured and 1/5th volume 5% CTAB solution was added and mixed well by gentle inversion . Equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged for 5minutes at 10,000rpm. Using a cut yellow tip transfer the top (aqueous) phase was transferred to a fresh tube and equal volume of CTAB precipitation buffer was added .The precipitated nucleic acid was spooled out, dissolved in high salt TE buffer, washed twice in 95% and 70% ethanol, dried and dissolved in 20µl of 0.1x TE (1mM TRIS, pH 8.0% and 0.1 mM EDTA, pH 8.0) buffer. The RNA was removed by RANse A treatment at 37^0c for 5minutes. Quantification of DNA was accomplished by using UV-VIS spectrophotometer (UV-1201 Schimazu corp.; Japan) and by analyzing the purified DNA on 1.5% agarose gels along side diluted double digest ECORI, Hind III double digest λ DNA as standard. DNA samples of 47 genotypes ware diluted in 0.1X TE to a concentration of approximately 100ng/µl for use in PCR analysis.

DNA amplification

Thirty decamer primers from Bangalore Genei Pvt. Ltd. (Table 5) were procured and each of the primers was dissolved in nanopure water to a concentration of 100 ng/µl. Amplification was carried out in a 25µl reaction volume containing 1X PCR buffer (10mM Tris-HCl, pH 8.0; 50mM KCl; 0.5mM $MgCl_2$), 200µM each of dNTPs, 100 ng of primer, 1 unit of Taq DNA Polymerase ($3U\mu l^{-1}$) and 100 ng of DNA template. The amplification was carried out in a thermal cycler (MJ Research, Inc., Model PTC-100™). The first cycle consisted of denaturation of template DNA at 94°c for 3 minutes, primer annealing (36°c) for 1 min and primer extension (72°c) for 3 minutes. In the next 43 cycles, the period of denaturation was reduced to 15 sec, while the primer annealing and primer extension time remained same as in the first cycle. The last cycle consisted of only primer extension (72°c) for 5 minutes.

PCR products were separated on a 1.5 % agarose gel containing ethidium bromide using 1X TBE buffer. The size of the amplified fragments was determined by using marker (Eco R I, Hind III double digest λ DNA). DNA fragments were visualized under UV light and photographed using Pentax ME Super camera with red filter. These photographs were used to score the DNA bands for analysis.

Analysis of DNA finger print pattern

The gels were scored for computer analysis on the basis of the presence or absence of the amplified products. If a product was present in a genotype, it was designated as '1' and if absent; it was designated as '0'. A total of 334 RAPD bands were scored in this way for the 30 primers used. The RAPD data was analyzed using NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System) computer package (Rohlf, 1990). The data were used to generate Jaccard's similarity coefficients for RAPD bands. Jaccard's similarity coefficients between each pair of accessions were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Average (UPGMA).

RESULTS

D^2 ANALYSIS

The aggregate effect of all the 19 characters tested by Wilk's criterion indicated significant difference among the varieties (χ^2 for 874 df =3017.029). Thus it would be worthwhile to classify the population in to different genetic groups on the basis of the characters chosen. In the present study divergence was assessed by Mahalanobis's D^2-statistic.

Following Mahalanobis' D^2 statistics, D^2 values were computed for 1081 possible paired combinations among the 47 genotypes in respect of 19 characters. The D^2 values ranged from 65.66 to 9350.30 indicating a substantial variation among the genotypes .It was found that while some genotypes were genetically close to each other, some were very widely divergent. The lowest D^2 value 65.66 between Thakurabhog and Ganjam Local 1, both local aromatic rice varieties indicated the genetic closeness between the local types. The other genetically close combinations were observed between Thakurabhogh and Tulasiphulla (91.73) and Lektimachi and Parbatjira (97.98). All these varieties were short grained local aromatic rice varieties.

The highest D^2 value (9350.50) and hence the greatest diversity was found between Jhingisiali, a local aromatic rice variety and Pusa Basmati 1, the long grained semi-dwarf basmati type aromatic variety. The other divergent combinations were Jhingisiali and Kernal Local (8534.43) and Nalidhan and Pusa basmati 1(8528.6). In all these cases, it was found that genetic divergence was maximum between local non-basmati aromatic and basmati type aromatic varieties.

Clustering pattern of the genotypes

All the 47 genotypes were grouped into six clusters following Tocher`s method (Table 2). The cluster I was the largest one containing 36 genotypes. Infact, the cluster contained all the local short grained aromatic rice varieties excepting Dubraj and Mahulakuchi. Among the local aromatic rice varieties, Dubraj was having the longest kernel length and Mahulakuchi the shortest. As kernel length was having the largest contribution towards divergence (28.4%), both the shortest and longest kernel types were eliminated from the cluster.

Cluster II contained three non-aromatic varieties like IR 64, Lalat, PR 106 and three basmati type aromatic varieties like Sanwal basmati, Basmati 370 and Taroari basmati possibly because of their longer kernel length.

Cluster III included one local aromatic widely grown rice variety (Dubraj) and another non-aromatic high yielding variety (Samba Mahsuri). Both of these varieties were clubbed because of their more or less similar grain types.

Cluster V containing semi-dwarf basmati type aromatic rice variety Pusa Basmati 1 was characterized by highest kernel length after cooking, alkali spreading value, water uptake and amylose content, but lowest kernel breadth.

Cluster VI containing Kernal Local was characterized by longest panicle, highest L/B ratio and volume expansion ratio. How ever it showed lowest expression for grain yield per plot, number of fertile grains per panicle hulling percentage and head rice recovery percentage.

Table 2 : Clustering pattern of 47 aromatic rice genotypes

Cluster	No.of genotypes	Names of genotypes
I	36	Badsabhog, Barangmali,Barikunja,Basanasapuri,Basuabhoga, Chhatianaki,Dhoiabankoi,Dubrajsena,Jala,Jalaka,Jhillipanjari, Jhingisiali,Kalajira,Kalajiri,Karpurakali,Kalikati,Khosakani, Kalakrushna,Lektimachi,Manasi,Leelabati,Nalidhan,Parijatak, Ratnasundari,Sirimula,Sujata,Thakurabhog,Thakurasuna, Parbatajira,Tulasiphulla,Tulasibasa,Durgabhog,Pimpudibasa, Nanu,Ganjam local 1,Ganjam local 2.
II	6	IR 64, Lalat, PR 106, Sanwal Basmati , Basmati 370, Taroari Basmati
III	2	Dubraj, Samba mahsuri.
IV	1	Mahulkuchi
V	1	Pusa Basmati 1
VI	1	Karnal Local

Clustering pattern and geographic origin

It is interesting to note that all but two local land races from Orissa formed a separate cluster (cluster I). But such constellations were not found in genotypes in cluster II and cluster III. However, there remains an indication of correspondence between genotype origin and genetic diversity.

Contribution of characters to genetic divergence

The relative contribution of different characters to genetic divergence was presented in Table 3. It was found that kernel length had the highest contribution (28.4%) to total divergence followed by water uptake (26.83%) and days to 50% flowering (19.61%). On the other hand, panicle length, panicle number, hulling %, milling %, L/B ratio, volume expansion, plot yield and elongation ratio had very insignificant contribution towards genetic divergence.

Table 3: Contribution of various characters towards genetic divergence

Characters	No. of times ranked first	Contribution(%)
Days to 50% flowering	212	19.61
Plant height(cm)	49	4.53
Panicle length(cm)	0	0.00
Panicle number	0	0.00
No. of fertile grains/panicle	16	1.48
100-grain weight(g)	52	4.81
Plot yield(kg/ha)	2	0.19
Hulling %	0	0.00
Milling %	0	0.00
Head rice recovery %	65	6.01
Kernel length(mm)	307	28.40
Kernel breadth(mm)	16	1.48
L/B ratio	2	0.19
Kernel length after cooking(mm)	13	1.20
Elongation ratio	3	0.28
Alkali spreading value	33	3.05
Water uptake(ml)	290	26.83
Volume expansion	2	0.19
Amylose %	19	1.76

Table 4 : Cluster means of 47 genotypes for 19 characters in aromatic rice

Characters	Average cluster means					
	I	II	III	IV	V	VI
Days to 50% flowering	111.5	110.2	114.0	102.5	108.5	93.5
Plant height(cm)	118.6	93.2	96.5	88.4	85.4	110.4
Panicle length(cm)	23.6	23.3	20.0	20.9	21.6	23.7
Panicle number	6.87	7.33	7.9	5.9	6.6	7.8
No.of fertile grains/panicle	87.8	72.9	106.8	75.4	42.0	36.5
100-grain weight(g)	1.28	2.2	1.6	1.4	1.9	1.8
Plot yield(kg/ha)	1999.4	3324.5	3494.4	1451.5	1520.8	1329.6
Hulling %	78.7	76.7	77.5	77.3	77.8	76.7
Milling %	73.6	70.4	72.3	72.2	71.9	71.3
Head rice recovery %	54.5	41.9	50.8	46.0	43.2	31.9
Kernel length(mm)	4.2	6.8	5.3	3.7	7.4	7.6
Kernel breadth(mm)	2.1	1.9	1.8	2.1	1.8	1.8
L/B ratio	2.0	3.5	2.8	1.8	4.2	4.2
Kernel length after cooking(mm)	8.0	12.5	8.8	9.3	15.2	13.4
Elongation ratio	1.9	1.8	1.7	2.5	2.0	1.8
Alkali spreading value	2.5	4.5	4.0	3.0	6.0	3.5
Water uptake(ml)	138.3	224.6	195.7	137.5	330.0	182.5
Volume expansion	3.9	4.1	3.8	5.0	4.3	5.1
Amylose %	20.8	22.9	22.6	22.2	23.1	20.3

RAPD Analysis

Thirty decamer primers were used in RAPD analysis of 47 rice genotypes. Each of the 30 primers varied greatly in their ability to resolve variability among the accessions. A total of 334 amplification products were obtained using these arbitrary primers across 47 genotypes. Out of 334 bands scored, 224 (67.07%) were found to be polymorphic and the rest were monomorphic. The number of bands per primer ranged from three (OPA 16) to 18 (OPK 15), the average bands per primer being 11.13 (Table 14). The size of the amplified product varied from

300 bp to 3500 bp. Maximum numbers of polymorphic bands (16) were obtained with the primers OPA 19 and OPF 17 and minimum (1) with primer OPI 6 (Table 6). The average number of polymorphic bands per primer was 7.46. Some of the primers showing good polymorphism were presented in Fig. 2 to Fig.6.

Table 5 : Primers used for generating RAPDs in aromatic rice germplasm

Sl.No.	Primers	Sequence(5/-3/)
1	OPA13	CAGCACCCAC
2	OPA16	AGCCAGCGAG
3	OPA17	GACCGCTTGT
4	OPA19	CAAACGTCGG
5	OPB8	GTCCACACGG
6	OPB11	GTAGACCCGT
7	OPB13	TTCCCCCGCT
8	OPB18	CCACAGCAGT
9	OPC9	CTCACCGTCC
10	OPE11	GAGTCTCAGG
11	OPE14	TGCGGCTGAG
12	OPF5	CCGAATTCCC
13	OPF6	GGGAATTCGG
14	OPF17	AACCCGGGAA
15	OPG16	AGCGTCCTCC
16	OPI6	AAGGCGGCAG
17	OPI16	TCTCCGCCCT
18	OPK4	CCGCCCAAAC
19	OPK6	CACCTTTCCC
20	OPK15	CTCCTGCCAA
21	OPL14	GTGACAGGCT
22	OPN1	CTCACGTTGG
23	OPN16	AAGCGACCTG
24	OPP2	TCGGCACGCA
25	OPP11	AACGCGTCGG
26	OPS19	GAGTCAGCAG
27	OPU13	GGCTGGTTCC
28	OPW2	ACCCCGCCAA

29	OPW7	CTGGACGTCA
30	jas 1.5	GAACGGACTC

Highly informative RAPD primers

Based on the level of polymorphism detected by individual primers, the ten most informative primers were OPA19, OPF17, OPB8, OPB13, OPI16, OPN1, OPU13, jas1.5, OPE14, and OPF 5. These primers amplified a total of 134 bands of which 115 (85.82%) were polymorphic. The primer OPA 13 amplified a slightly more than 900 bp band only in aromatic varieties. The primer jas 1.5 (Fig.4) amplified an around 600 bp band in some varieties.

Table 6 : Number of bands amplified by different primers in RAPD analysis

Sl.No.	Primers	Total No. of bands	No.of monomorphic bands	No. of polymorphic bands
1	OPA13	11	4	7
2	OPA16	3	1	2
3	OPA17	6	0	6
4	OPA19	15	0	15
5	OPB8	14	1	13
6	OPB11	7	1	6
7	OPB13	12	1	11
8	OPB18	12	4	8
9	OPC9	7	2	5
10	OPE11	7	2	5
11	OPE14	12	3	9
12	OPF5	13	4	9
13	OPF6	11	4	7
14	OPF17	16	1	15
15	OPG16	14	10	4
16	OPI6	11	10	1
17	OPI16	13	2	11
18	OPK4	13	7	6
19	OPK6	16	8	8

20	OPK15	18	10	8
21	OPL14	11	5	6
22	OPN1	11	0	11
23	OPN16	14	5	9
24	OPP2	7	6	1
25	OPP11	4	1	3
26	OPS19	13	6	7
27	OPU13	14	3	11
28	OPW2	5	0	5
29	OPW7	10	5	5
30	JAS1.5	14	4	10

Similarity Coefficients

Jaccard's similarity coefficients estimated on the basis of all the 30 primers ranged from 0.625) to 0.922 with a mean of 0.789. The short grained aromatic genotypes particularly Thakurabhog and Ganjam local 1(0.922) as well as Tulasibasa and Durgabhog (0.922) were highly similar. Maximum diversity was obtained between short grained aromatic variety Parijatak and non-aromatic variety Sambamahsuri (0.625).

Phylogenetic tree

A phylogenetic tree (Fig.1) generated using Unweighted Pair Group Method with Arithmetic Average (UPGMA) cluster analysis separated the 47 genotypes into two major groups (I and II). The first major group consisted of all the aromatic rice genotypes (local short grained aromatic varieties and basmati type long grained varieties) excepting Dubraj and Sujata; where as the non-aromatic varieties were included in the second major group. Each of these major groups was further sub-grouped. All the short grained local aromatic varieties excepting Dubraj and Sujata were clustered into one sub-group (IA1). The commercially important exportable long grained aromatic rice varieties such as Pusa Basmati 1, Taroari Basmati, Sanwal Basmati, Kernal Local and Basmati 370 were included in a major sub-cluster (IA2). Similarly all the non-aromatic high yielding varieties such as Lalat, PR 106, IR 64 and Sambamahsuri were clubbed together in a major sub-cluster (IIB).

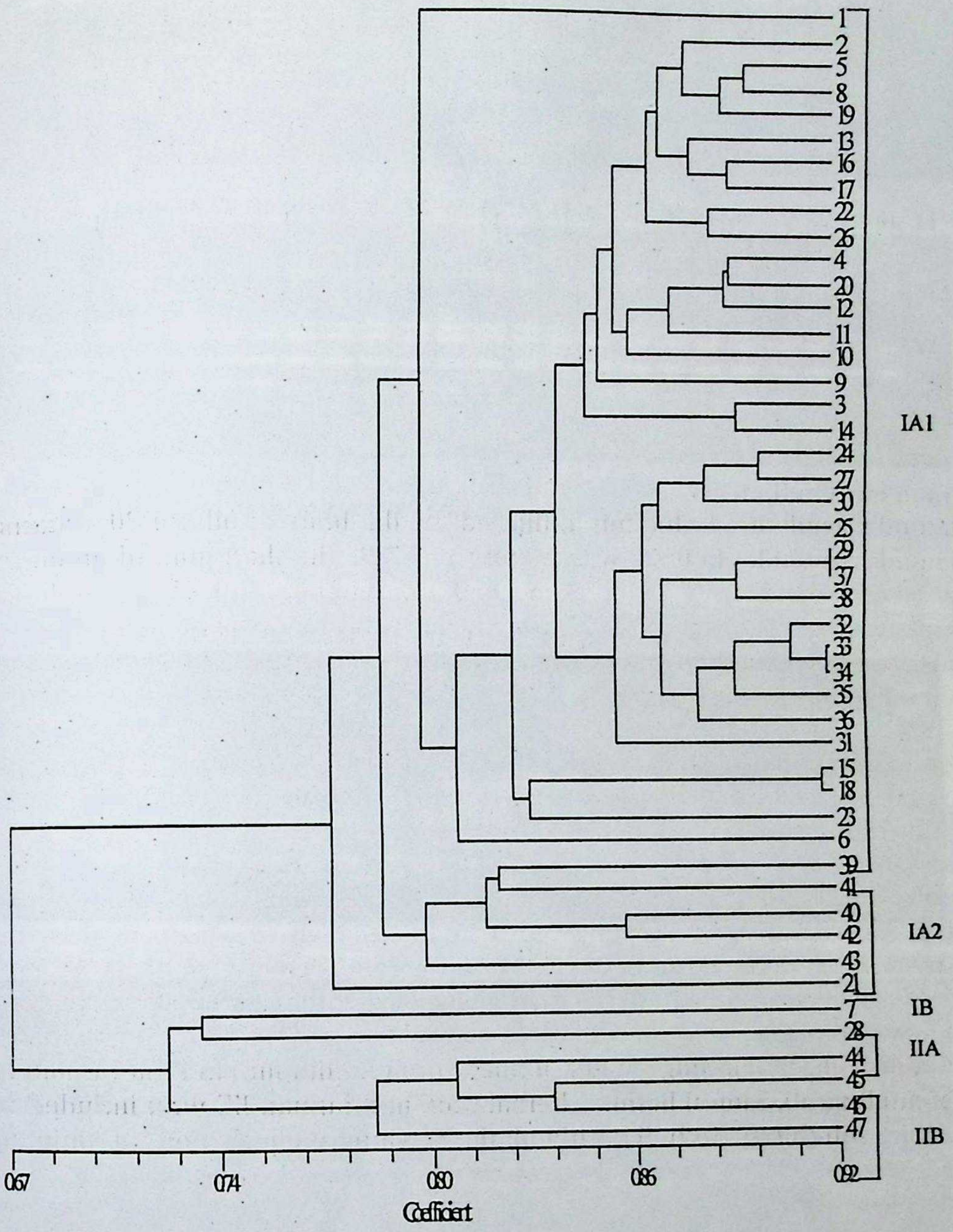

Fig. 1 : Dendrogram of the rice varieties constructed using UPGMA based on Jaccard's similarity coefficients. The major clusters and sub clusters are indicated on the right margin.

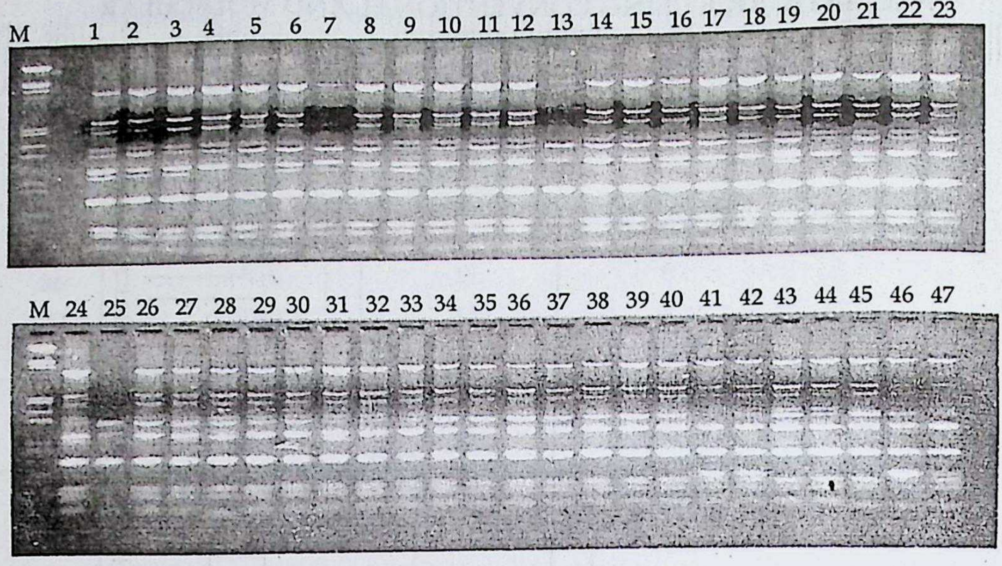
M
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
M
24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47

Fig.2 : RAPD profile of aromatic rice varieties with primer OPF 5

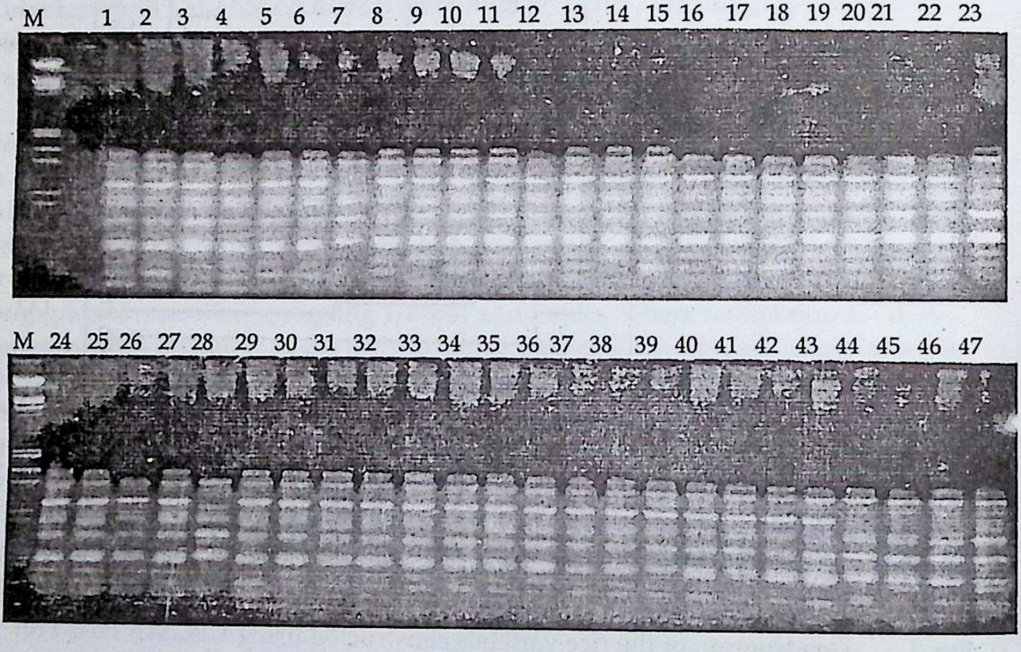
M
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
M
24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47

Fig. 3 RAPD profile of aromatic rice varieties with primer OPU 13

ASSESSMENT OF GENETIC DIVERSITY IN SHORT GRAINED AROMATIC RICE USING CONVENTIONAL AND MOLECULAR APPROACHES

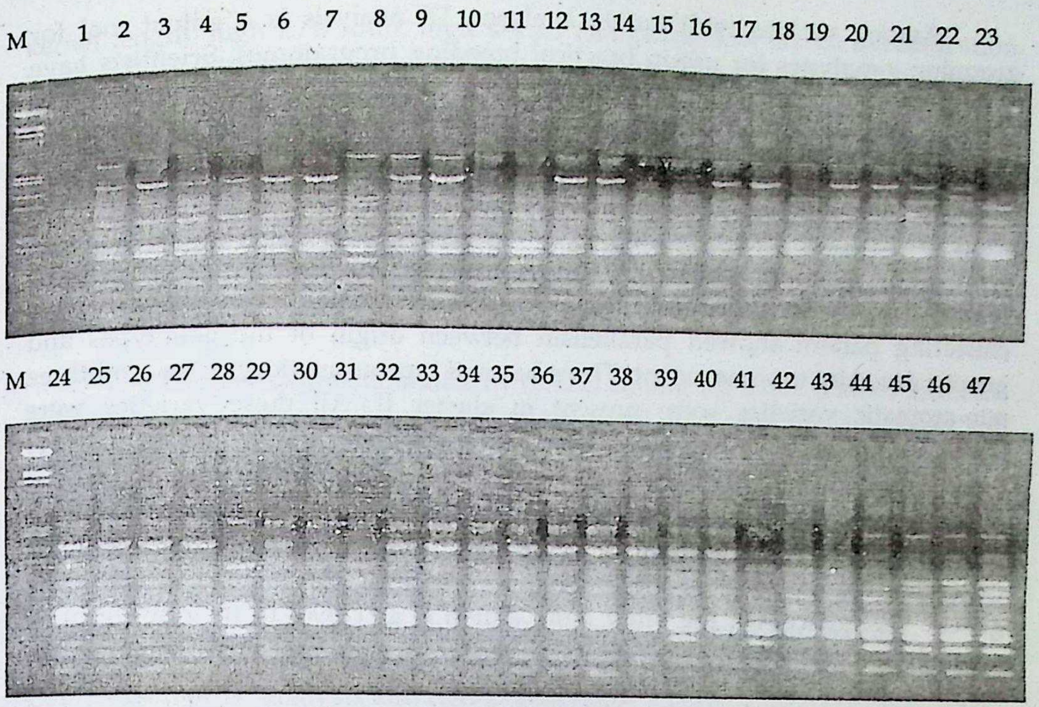
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
M 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47

Fig. 4 : RAPD profile of aromatic rice varieties with primer jas 1.5

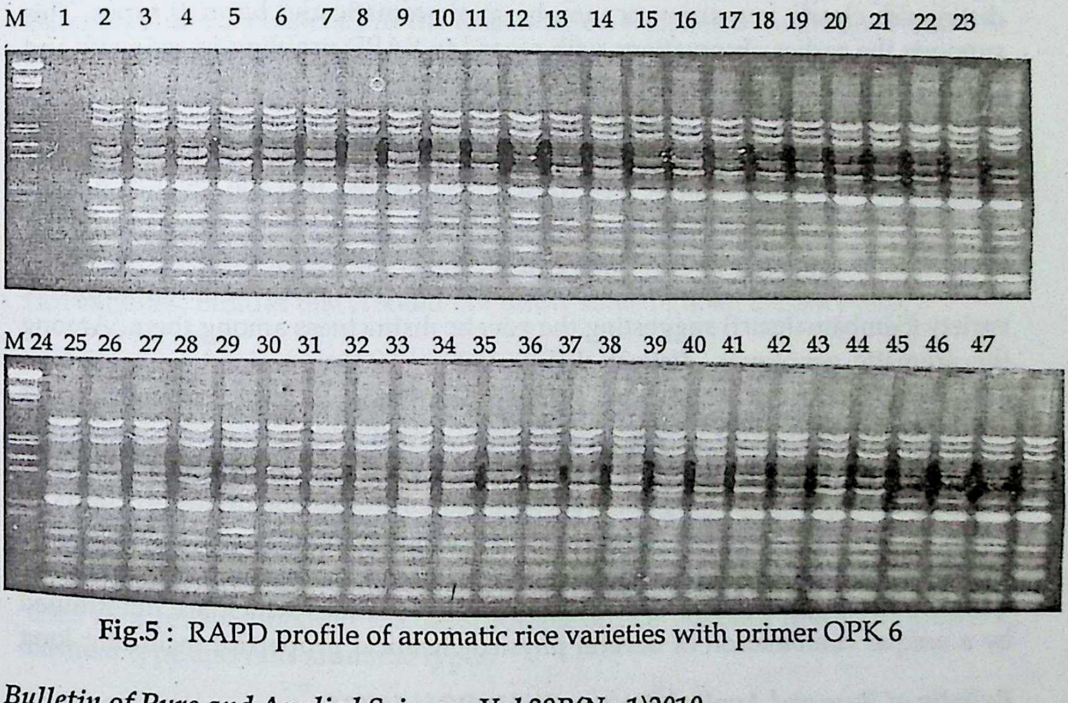
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
M 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47

Fig.5 : RAPD profile of aromatic rice varieties with primer OPK 6

DISCUSSION

Among various multivariate analyses, D^2 analysis is a potent tool for grouping genotypes for use in practical breeding programmes. Scientists have extensively used Mahalanobis' D^2 statistic in genetic grouping of germplasm in a large number of crop plants (Murty and Arunachalam, 1966; Ramanujam *et al.*, 1974 and Arunachalam, 1981). In the present investigation, the 47 varieties were grouped into six clusters including three mono genotypic clusters. All the local short grained varieties excepting two varieties, Dubraj and Mahulkuchi were grouped into the same cluster. Dubraj was having the longest kernel length and Mahulkuchi, the shortest kernel among the 38 local aromatic rice varieties. So the clustering pattern showed parallelism between origin of the genotypes and genetic diversity to some extent. Three basmati type aromatic varieties and three non-aromatic varieties were present in cluster II. All these varieties were clustered together apparently because of their long kernels. Kernel length, water uptake and days to 50% flowering were the main contributors to genetic divergence. Similar results were also reported by Ratho (1984), where all the 43 scented rice varieties were grouped into five clusters and kernel length, volume expansion ratio and the alkali spreading value were the main contributors to genetic divergence. On the other hand, Singh *et al.* (1996) in their study of 40 scented, fine grained rice varieties observed grain yield and plant height as the major contributors to total divergence.

In the presented study, RAPD profiling was found efficient enough to distinguish clearly non-aromatic, non-basmati aromatic and basmati types. This supports the earlier observations with regard to RAPD profiling in aromatic and non-aromatic rice (Baishya *et al*, 2000; Raychoudhury *et al*, 2001).

The average similarity index of 0.802 among the local scented varieties and 0.773 among the basmati type varieties suggested that the level of genetic diversity among the aromatic rices is low. Similar results were also reported by Raychoudhury *et al*, 2001. Minimum similarity coefficient (0.625) was found between a local short grained aromatic variety (Parijatak) and non-aromatic variety (Sambamahsuri) suggesting the genetic distinctness among them. Among the aromatic genotypes, distinct differentiation of long-grained basmati type aromatic rices from the short grained non-basmati local aromatic rices correspond well with their geographic distribution as well as grain characteristics. The traditional long grained aromatic rice varieties like Basmati 370, Kernal local, Taroari Basmati etc. are grown in the north-western parts of India, where as short-grained non-basmati aromatic rice varieties are grown mostly in eastern and north-eastern parts of India. The basmati types possess most desirable grain characteristics and cooking qualities which are determined by a unique combination of several physico-chemical properties including long kernel length, high L/B ratio, high kernel elongation, low starch gelatinization temperature, intermediate amylose content and medium gel consistency that are collectively called basmati traits .In contrast, the non-basmati aromatic varieties though possess strong aroma, lack in more desirable basmati traits.

The extent of genetic similarity as determined by RAPD finger printing also correspond considerably with the known pedigree. For instance, Taroari Basmati, which is a selection from Kernal local grouped together with its progenitor parent at similarity index of 0.858.

High degree of genetic similarity (0.922) among the short grained aromatic cultivars such as Thkurabhog and Ganjam Local 1 as well as Tulasibasa and Durgabhog indicated that they are possibly the selections made by farmers from a single land race.

On the basis of the observations in the present study, it is suggested that RAPD analysis can be efficiently used for the purpose of studying the genetic diversity, though it involves considerable amount of effort, time and cost.

Comparison of D^2 analysis and RAPD analysis

The Mahalanobis' D^2 analysis was done taking into consideration 19 morphological and physico-chemical characters. The 47 genotypes were classified into six clusters. All the local scented varieties were grouped in to the cluster I excepting Dubraj and Mahulkuchi. In the cluster II, three aromatic long grained basmati type varieties and three non-aromatic long grained varieties were present, while in cluster III, two medium grained varieties, one being aromatic (Dubraj) and another non-aromatic (Sambamahsuri) were clubbed together. Two basmati type aromatic varieties Pusa Basmati 1 and Kernal Local related by ancestry formed two distinct monogenotypic clusters. Thus D^2 analysis could not give a clear cut classification of aromatic and non-aromatic rice varieties. Besides that it could not throw some light on ancestry also.

On the other hand, from the dendrogram constructed based on RAPD data, at 75% similarity coefficient level, all the aromatic varieties were clubbed in to a single cluster excepting two local varieties Dubraj and Sujata. The non-aromatic varieties also formed a distinct cluster. At a higher similarity level, the local aromatic rice and basmati types formed two distinct sub-clusters.

Thus, from the present study it can be inferred that RAPD analysis can be used as a reliable method for classification of rice varieties into local aromatic, basmati type and non-aromatic types.

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INDUCTION OF CALLUS FROM DIFFERENT EXPLANTS IN STEVIA REBAUDIANA

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ABSTRACT

In the present studies callus was induced from different explants like the shoot apex, nodal region and leaf. The sterile explants were than transferred int MS. Media containing of BAP 0.5mg/l. callus was obtained from all explants and in case of nodal region multiple shoots are produced. Callus formation was observed earlier from the leaf segments than the nodal regions. The colour of the callus ranged from white to green. At the end of one week of sub-cultured explants the intensity of callus was increased and maximum callus was noticed. Multiple shoots are obtained from nodal region and from the callus formed near the nodal region also obtained the growth of multiple shoots through organogenesis.

Key words: Explants, Callus, Organogenesis.

INTRODUCTION

Stevia rebaudiana Bert, belonging to the family Compositae. It is commonly known as sweet leaf, sugar leaf or simply stevia. It is one of the most valuable tropical medicinal plants. With its extract having upto 300 times the sweetness of sugar, stevia has garnered attention with the rise in demand for low carbohydrate, low sugar food alternatives S.rebaudiana is originally a South American Wild plant (Katayma *et al.*, 1976),seed germination is poor (Miyazaki & Wantenabe,1974). Therefore there are two ways of Vegetative propagation; Conventional and non-conventional type.

It is small shrubby perennial plant growing upto 65cms height tall with sessile, oppositely arranged lanceolate to oblaceolate leaves, serrated above the middle. The property of the species that called attention to the plant was the intense sweet taste of the leaves and aqueous extracts. The first report of commercial cultivation in Paraguay was in 1964 (Ktayma *et al.*, 1976;Lewis,1992). From the leaves of stevia,stevioside, sweet crystalline diterpene glycosides are extracted.Pure extract is non caloric and 30 times sweeter than sugar (Bhosle,2004) .The product can be added to tea and ,cooked or baked goods, processed foods and beverages. Stevioside is of special interest to Diabetic persons with hyperglycemia and the diet conscious people.

MATERIALS & METHODS

The explant (leaf, nodal, internodal segments) were collected from the field plants of Stevia reaudiana . The eplants were chopped into small pieces (about 0.1m long) and then treated with 1% liquid detergent (tween 80) for 5-6 minutes with constant shaking and washed thoroughly with distilled water. Then the explants were surface sterilized with 0.001% Mercuric chloride solution for 2-3 minutes & the explants were then inoculated aseptically into MS.Medium (Murasshige & Skooge,1962) with different concentration & combinations of growth regulators (Table-1).

Table 1: Composition of MS. Media (1 litre)

CATEGORY	CHEMICALS	AMOUNT
Macro salts	NH_4NO_3	1.65g
	KNO_3	1.90g
	$CaCl_2.2H_2O$	0.44g
	$MgSO_4.7H_2O$	0.37g
	KH_2PO_4	0.17g
Micro salts	$FeSO_4.7H_2O$	27.80mg
	$Na_2EDTA_2H_2O$	33.60mg
	KI	0.83mg
	H_3BO_4	6.20mg
	$MnSO_4.4H_2o$	22.30mg
	$ZnSO_4.7H_2O$	8.60mg
	$Na_2MOO_4.H_2O$	0.25mg
	$CuSO_4.5H_2O$	0.025mg
	$CoCl_2.6H_2O$	0.025mg
Organic supplements	Myoinositol	100.00mg
	Nicotinic acid	0.05mg
	Pyridoxine HCL	0.05mg
	Thiamine HCL	0.05mg
	Glycine	0.02mg
	Sucrose	30.00mg

After mixing all the salts & organic components for 500ml media to which 4gm of Agar & 15gm of Sucrose was mixed to solidify it then the hormone BAP.0.5mg/l is added Total media was sub divided into 500 ml conical flask &

500 ml of distilled water along with culture tubes 7 cotton plugs were autoclaved at 121^{0}C temperature & 15 Psi or 20min.Then the media was divided into 25 culture tube & placed in vertical tube stand. Next day it was inoculated.

Callus Induction :-

Ms. Media with all the stock solution along with 0.5mg/l and 0.1 mg/l BAP was taken as shown in the Table 2.which was autoclaved & Poured into the culture tubes & on the next day the leaf , nodal & inter nodal segment were inoculated & from 4th day onwards callus induction was seen on the leaf & on 18th day in nodal region it was observed.

Table 2: Production of Stevia rebaudiana callus in different concentrations of harmones

Harmones BAP	Explant		
	Leaf	Node	Internode
0.5	+ + +	+ + +	+ +
0.1	+ +	+ + +	+

+ Poor Callus, + + - Medium Callus , + + + - Profuse Callus

The colour of the callus ranged from White to Green .At the end of one week of sub culture explants the intensity of callus was increased and maximum callus was noticed. Multiple shoots are obtained from nodal region and from callus formed near the nodal region also obtained the growth of the multiple shoots through Organogenesis. Unless mentioned specially all cultures were grown in an air-conditioned culture room illuminated by 40 W white fluorescent tube. The temperature of the culture room was maintained around 25° C. Visual observation was made daily and developmental changes were noticed.

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CONCLUSIONS

Among all the explants used for the culture the leaf and Nodal region showed the formation of the callus and multiple shooting and Organogenesis were seen which indicate the possibility of propagating of S.rebaudiana is possible in invitro condition with all its potentiality and suitability.

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EFFECT OF PRE-PLANT HERBICIDES ON WEED CONTROL AND PERFORMANCE OF OKRA *ABELMOSCHUS ESCULENTUS* IN A SOUTH WESTERN NIGERIAN LOCATION.

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ABSTRACT

Field trials were carried out over two years to evaluate the performance of Okra *Abelmuschus esculentus* and weed control efficacy using pre-plant herbicides. The treatments were weedy check, pre-plant glyphosate, pre- plant pendimethalin at three different applications of 10, 7, and 3days before planting, and hoe weeding. Pendimethalin applied pre-plant either at 3, 7 or 10DBP at the rate of 1.0kg active ingredient ha^{-1} and hoe weeding twice at 3 and 5weeks after planting produced better weed biomass reduction, plant height, and pod yield when compared with the pre-plant glyphosate application and the weedy check control. Pre-plant pendimethalin at 3DP gave slight reduction in emergence and subsequent establishment counts.

Key words : *Abelmuschus esculentus*; Glyphosate; Pedimenthalin; Hoe weeding; Weedy check control.

INTRODUCTION

Okra *Abelmoschus esculentus* is an important vegetable crop that is widely grown in the tropics. The matured fruits are used as boiled or fried vegetable. They are particularly popular for adding to soups and stews either in fresh form or dried powdered flour form (Ariyo and Akenova,1986).It has been observed that large scale okra production is hampered by diseases and weeds with weeds being responsible for about 30%of the crop loses (Chew and Yeong 1977). Adeponwo (1993) had reported a pod yield reduction of up to 89% due to uncontrolled weeds in sole cropped rain fed okra in the humid zone of south western Nigeria. Herbicidal weed control had been reported in different places resulting in effective weed control that produce good okra yield comparable with those of laborious hand weeding (Nandal and Sighn 1993). Little attention had been paid to the use of herbicide for effective weed control in okra in Ekiti State of Nigeria. The present study therefore aimed at studying the effect of two pre-plant herbicides in comparison with the traditional hand weeding in weed control for optimum okra production in Ekiti State location of Southwestern Nigeria.

MATERIALS AND METHODS

The experiment was conducted at the experimental site of the Department of Plant Science of the University of Ado-Ekiti, Nigeria (8^0 45^1N 5^0 15^1E). The site has a bimodal annual rainfall mean of about 1350mm. The soil at the site was a sandy loam with an organic matter content of 4.2% and4.4%; 0.21% and 0.23% Nitrogen; 3.75mg/kg and 3.71mg/kg Phosphorus; 198ppm and 197ppm Potassium and pH of 6.9 and 6.5 respectively in 2004 and 2005. The soil is a sandy clay alfisol (60% sand, 22%clay, and 18% silt). The field had been under *Tithornia diversifolia* (L) and *Chromolaena odorata* (L) for two years.

The experiment was laid out in a randomized complete block replicated four times. The site was manually and ridged with each plot measuring 6 by 4metres. Six weed control treatments were employed: weedy check (WC) as control; hoe weeding (HW); pre-plant Glyphosate (GP); pre-plant pendimethalin (PM) at 10, 7 and 3 days before planting (WBP).Glyphosate and Pendimethalin were applied at the rate of 2.4kg and1.0kg active ingredient per hectare [a.i.ha^{-1}] respectively. Hoeing was done at 3and 5 weeks after planting (WAP).

Three seeds of okra V35 variety collected from Ekiti state Agricultural impute supply Ado-Ekiti were sown and later thinned to one plant per hill at 2WAP. Planting was done in May and October of 2004 and 2005 respectively. Emergence count of okra seedlings was taken at 10days after planting while the final plants stand count was taken at 5WAP. Weed control assessment was carried out by sampling weeds using 1m^{2}quandrat randomly placed on each plot at 4WAP.

Plant height was taken at 4WAP. Yield and yield attributing factors were assessed from five randomly selected plants per plot. All data were subjected to statistical analysis using the Duncan's multiple range tests.

RESULTS AND DISCUSSION

The predominant weeds noticed in the two years were *Euphorbia heterophylla* (L) *Tithornia diversifolia* (L) and *Panicum maximum* Jacq. The results obtained from this study show that okra seedlings emergence and final plant establishment counts were identical in all the weeded plots except in the pendimethalin at 3DBP where lower emergence and final plant counts were observed (Table1). Lower emergence percentage and lower final plant count observed in the 3DBP pendimethalin applied plots probably reflects higher concentration of the herbicide not yet dispersed in the soil environment where the okra seeds were germinating. The chemicals were probably yet to dissipate properly within the short period of application before okra seeds were planted.

Table2 shows the effect of the weed control treatments on plant height and weed biomass. All the pendimethalin applied plots gave effective weed biomass reduction comparable to those of hoe weeding in both seasons. Glyphosate was not as effective as pendimethalin and hoe weeding. However, weedy check control gave the highest weed biomass. The reason for poorer weed biomass reduction in glyphosate applied plots than pendimethalin can be attributed to the fact that glyphosate is a systemic contact herbicide that has no phytotoxic effect on weed seeds that germinated after application. Pendimethalin on the other hand is a soil acting herbicide that kills all germinating seeds in the soil. Taller plants recorded in pendimethalin applied plots than the glyphosate applied plots reflects the reduced effect of weed on okra in the pendimethalin plots as observed in weed biomass assessment.

Yield and yield component assessment revealed that all the herbicides used in this study effectively promote better okra yield than the weedy check control plots (Table3). Number of fruits per plant as well as pod lengths was identical in all the herbicide applied plots and hoe weeded plots. Fruit yield was lowest in the weedy check plots. All the pendimethalin applied plots gave good yields comparable to those of hoe weeding. There were 80.5 and 81.8% yield losses in 2005 and 2006 seasons respectively in the weedy check control plots when compared with hoe weeding.

It is clear from the results obtained from this trial that best weed control methods that reduced weed biomass also gave the highest okra yield pre-plant. Rogers and Talbert 1979 had reported the effectiveness of glyphosate in reducing weed growth to an acceptable level and subsequently increased okra yield. The present trial showed that pendimethalin applied at 7DBP or 10DBP gave maximum field establishment of okra, reduced weed biomass and gave the highest yield as did the hoe weeded plots. Rajagopal and Ashokmetha 1997, and Nandal and Sighn 1993 had reported similar efficacy of pre-emergence herbicidal weed control in okra which resulted in increase yield. However Sandhu and Tariok (1991) had reported a supplementary hand weeding at 6WAP to pre-emergence application of fluchloalin and pendimethalin to produce the highest yield when compared with 2 hand weedings and unweeded controls.

It is suggested that pedimenthalin be applied to the soil and left to work itself through the soil to effectively eliminate weed seeds and reduce lethal effect on okra. This is because the 3DBP pedimenthalin application led to a slight reduction in seedling emergence and field establishment count.

It is also to be noted that a single dose of herbicide application in this trial compared very well with the 3 and 5WAP hoe weeding in reducing weed infestation and subsequent yield increase. It had been noted that the cost of a single hoe weeding is about ten times the cost of herbicide application.

Table 1 : Emergence count (%) and establishment count (%)

Treatment	Emergence% 2004	Emergence% 2005	Establishment count 2004	Establishment count 2005
Weedy check	96.0a	97.0a	85.0a	87.0a
Hoe weeding	97.5a	96.9a	95.0a	94.0a
Glyphosate	98.0a	96.5a	97.0a	95.0a
Pendimethalin at 10DBP	97.5a	98.0a	97.5a	97.0a
Pendimethalin at 7DBP	96.5a	97.0a	96.5a	97.0a
Pendimethalin at 3DBP	74.5b	76.0b	75.0b	71.0b

Means with the same letter(s) within columns are not significantly different (P=0.05)

Table 2: Plant height and weed Biomass with the different weed control methods

Treatments	weed biomass(gm^2) 2004	weed biomass(gm^2) 2005	Plant height(cm) 2004	Plant height(cm) 2005
Weedy check	251.6a	233.5a	20.1c	21.3c
Hoe weeding	5.4c	5.1c	27.5a	26.0a
Glyphosate	23.5b	20.3b	24.2b	23.8b
Pendimethalin at 10DBP	8.4c	9.0c	27.0a	27.5a
Pendimethalin at 7DBP	5.1c	4.5c	26.4a	26.0a
Pendimethalin at 3DBP	4.0c	3.1c	26.5a	26.0a

Means with the same letter(s) within columns are not significantly different at (P=0.05)

Table 3 : Yield and yield components with different weed control methods

Treatments	Fruit yield(gm/plant)		No of fruits/plant		Pod length(cm)	
	2004	2005	2004	2005	2004	2005
Weedy check	121.0c	129.0c	2.1c	3.1c	3.6c	4.1c
Hoe weeding	620.2a	710.4a	17.5a	18.0a	9.1a	8.8a
Glyphosate	583.5b	580.2b	16.5a	17.0a	7.7b	7.9b
Pendimethalin at (10DBP)	598.7ab	698.4a	18.0a	17.3a	8.9a	8.7a
Pendimethalin at (7DBP)	613.6a	709.6a	19.9a	17.0a	9.0a	8.8a
Pendimethalin at (3DSP)	610.0a	699.8a	17.1a	16.5a	9.2a	8.7a

Means with the same letter(s) within columns are not significantly different at (P=0.05)

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AEROMYCOFLORA OF A COLLEGE LIBRARY IN IMPHAL

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ABSTRACT

$Monitoring of airborne fungal spores was carried out inside the library of Standard College, Imphal for two years (January 2007 – December 2008) using Rotorod Air Sampler. Altogether 26 fungal types were identified out of which Aspergilli-Penicilli ranked highest contributing 21.23% of the total population. Known biodeteriogenic and allgergenic fungal types like *Cladosporium, Penicillium, Curvularia, Alternaria, Chaetomium, Rhizopus, Cunninghamella,* etc. were detected. Quantitative seasonal variation of fungal spores was observed to be influenced by physical and climatic factors in addition to available substrates.

Key words : Library, Airborne Fungi, Biodeterioration, Allergen.

INTRODUCTION

The deterioration of library materials (books, journals, scripts, note sheets, documents etc.) by microorganisms are well known and attracted the attention of many workers in the recent years. Microorganisms like fungi, bacteria and actinomycetes are referred to as biodeteriorating agents in libraries (Greathouse, 1950; Gallow, 1963). Further human exposure to airborne microorganisms, results in allergic and irritant responses, respiratory problems and hypersensitivity reactions (Agarwal and Shivpuri, 1974; Tilak, 1989; Apetrei *et. al.*, 2009).

Even though the deterioation of paper by the activity of microorganisms in India was known as early as 1960 by Kathapalia (Kathapalia, 1960), the studies on airspora inside library was first carried out in India by Tilak and Vishwe (1975). They showed the relation between the spore concentration in the air of library and book deterioration. Since then, many workers studied the microorganisms present inside library environment (Vittal and Glory, 1985; Sahne and Purwar, 2001; Atluri and Padmini, 2002; Majumdar and Hazara, 2005; Upadhyaya and Jain, 2005). However, information about the airspora inside library is very scanty in Manipur except Devi, 1998 and Devi and Singh, 2007. Hence, the present study on airspora inside the library and its working environments was undertaken to understand the components of airspora, seasonal variations, effect of meteorological parameters, relationship with sources, etc.

Table 1 : Percentage contribution of different spore types to the total fungal airspora from the indoor air of a College Library in Imphal.

* excluding hyphal fragments.

Sl. No.	Spore types	Percentage Contribution		
		2007	2008	Total of 2007 & 2008
	MASTIGOMYCOTINA	-	-	-
	ZYGOMYCOTINA			
1	*Cunninghamella*	0.24	025	0.49
2	Round spores (*Rhizopus-Mucor* type)	0.46	0.44	0.90
		0.70	0.69	1.39
	ASCOMYCOTINA			
1	Fusiform ascospores	0.56	0.59	1.15
2	*Chaetomium*	0.37	0.39	0.76
3	*Pleospora*	0.09	0.12	0.21
		1.02	1.10	2.12
	BASIDIOMYCOTINA			
1	Basidiospores	0.45	0.38	0.83
2	Uredospores	0.29	0.37	0.66
		0.74	0.75	1.49
	DEUTEROMYCOTINA			
1	*Alternaria*	4.15	5.80	10.55
2	Aspergilli-Penicilli	10.01	11.22	21.23
3	*Cladosporium*	8.30	8.01	16.31
4	*Colletotrichum*	0.55	0.33	0.88
5	*Corynespora*	0.61	0.45	1.06
6	*Curvularia*	3.73	3.75	7.48
7	*Diplodia*	0.03	0.05	0.08
8	*Drechslera*	1.68	1.74	3.42
9	*Epicoccum*	0.75	0.72	1.47
10	*Fusarium*	3.50	3.38	6.88
11	*Helminthosporium*	0.77	0.84	1.61
12	*Nigrospora*	2.63	2.77	5.40
13	*Periconia*	0.36	0.45	0.81
14	*Pestalotiopsis*	0.29	0.20	0.49
15	*Pithomyces*	0.28	0.18	0.46
16	*Spegazzinia*	0.09	1.01	1.10
17	*Tetraploa*	0.12	0.13	0.25
18	*Torula*	0.59	0.63	1.22
19	*Trichothecium*	0.23	0.17	0.40
		39.27	41.83	81.10
	OTHER TYPES			
1	Hyphal fragments	3.13	3.16	6.29
2	Others *	3.60	3.99	7.59
		6.73	7.15	13.88
	GRAND TOTAL	48.47	51.53	100

Table 2 : Monthwise fungal spore density and percentage contribution to the indoor air of a college library in Imphal.

Months	Total No. of spores		% of yearly total		Total No. of spores	% of two yearly total
	2007	2008	2007	2008	2007+2008	2007+2008
Jan	3250	3005	3.05	2.82	6255	5.87
Feb	4125	3770	3.88	3.54	7895	7.42
Mar	3160	3265	2.97	3.07	6425	6.04
Apr	4420	2995	4.15	2.81	7415	6.96
May	3450	3685	3.24	3.46	7135	6.70
Jun	4045	5035	3.80	4.73	9080	8.53
Jul	5010	4160	4.71	3.91	9170	8.62
Aug	6840	6830	6.42	6.41	14990	10.84
Sep	4715	6215	4.43	5.84	10930	10.27
Oct	4340	8150	4.07	7.66	12490	11.73
Nov	4570	4220	4.29	3.96	8790	8.25
Dec	3670	3515	3.45	3.30	7185	6.75
Grand Total	51595	54845	48.47	51.53	106440	100

MATERIALS AND METHODS

Monitoring of airborne fungal spores was carried out in the indoor environment of Standard College, Imphal for a period of two years (January, 2007 to December, 2008) using Tilak rotorod air sampler. The sampler was operated at weekly intervals for half an hour, twice a day (10.30 – 11 a.m. and 3.30 to 4 p.m.). Transparent cellotape was applied to the rods, trimmed back to the width of the rod with a sharp razor blade and then coated with vaseline. Then the air was sampled by operating rotorod sampler kept at 1 metre above ground level. After operating, the cellotape was removed and mounted beneath a cover glass using glycerine jelly and thus prepared the slides. Scanning of the prepared slides was done regularly throughout the investigating period. Identification of fungal spores was based on morphological characters, visual identification by comparison with reference slides and published literatures (Ellis, 1971; Barnett and Hunter, 1972; Hawkswork *et. al.*, 1985). Spore counts were converted to number per cubic meter of air by multiplying with a conversion factor of 5. Meteorological data during the study period was obtained from meteorological section of ICAR Research Complex, Lamphelpat, Imphal.

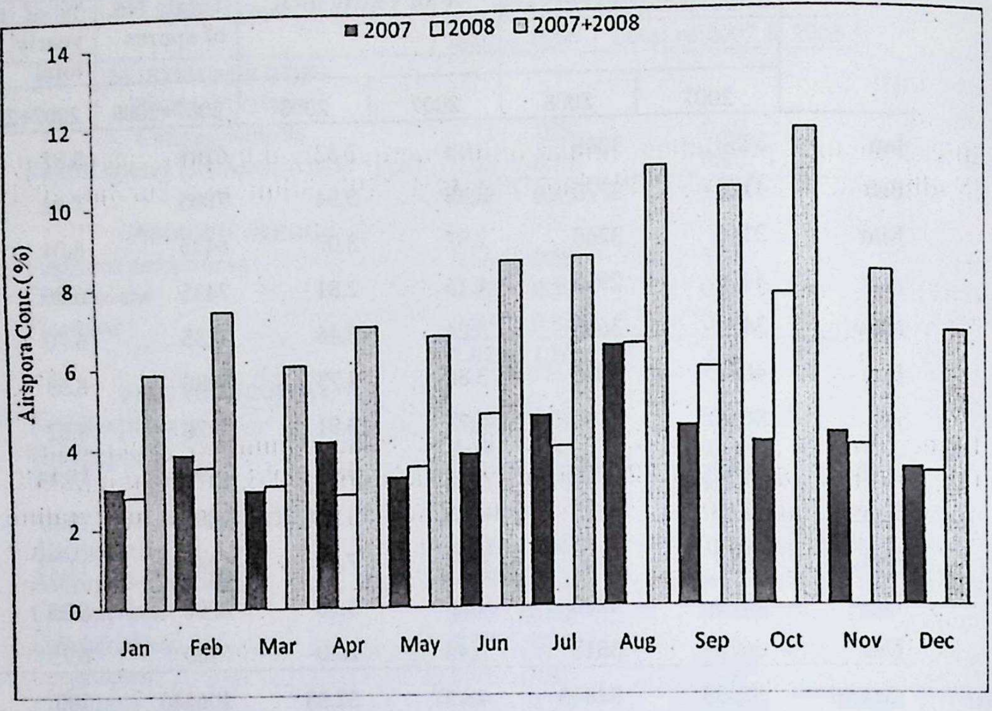

Figure 1 : Monthly variation of total indoor fungal airspora of Library for 2007, 2008 and sum of 2007 & 2008.

RESULTS AND DISCUSSION

A total of 1,06,440 spores/m^3 of air were trapped and assigned to 26 fungal types excluding hyphal fragments, insect parts, epithelial tissues, algal fragments, pollen grains, unidentified types etc. The trapped spores were put under two main groups – fungal spores and other types. Fungal spores contributed 86.12% and other types contributed 13.88% of the total population. The fungal spores identified were classified into five fungal subdivisions following standard literatures (Ainsworth, 1966; Hawkswork *et.al.,* 1985). Airborne fungal spores were present throughout the study period. Table I revealed 28 spore types identified from the indoor air of library, out of which 19 fungal types belonged to Deuteromycotina, the dominant types being *Alternaria,* Aspergilli-Penicilli, *Cladosporium, Curvularia, Drechslera, Fusarium, Nigrospora, Torula,* etc.; 3 to Ascomycotina as fusiform ascospores, *Chaetomium* and *Pleospora;*

2 to Basidiomycotina as basidiospores and uredospores; 2 to Zygomycotina as *Cunninghamella* and round spores (*Rhizopus-Mucor* type) and nil to Mastigomycotina. Deuteromycotina ranked the highest (39.27% in 2007 and 41.83% in 2008) which was followed by Ascomycotina (1.02 and 1.10), Basidiomycotina (0.74 and 0.75) and Zygomycotina (0.70 and 0.69). From the above analysis, it was clear that Deuteromycotina contributed more than 80% of the total population whereas the remaining sub-divisions (Ascomycotina, Basidiomycotina, Zygomycotina and Mastigomycotina) contributed less than 20% of the total population. Similar results were reported by previous workers (Nadimuthu and Vittal, 1995; Saoji and Giri, 1997; Sahne and Purwar, 2001; Upadhyaya and Jain, 2005). Some of the fungi having high frequency of occurrence were Aspergilli-Penicilli (21.23%), *Cladosporium* (16.31%), *Alternaria* (10.55%), *Curvularia* (7.48%), *Fusarium* (6.88%), hyphal fragments (6.29%), *Nigrospora* (5.40%) and *Drechslera* (3.42%).

Seasonal variation in concentration of spores was observed. During 2007, the highest (2.30%) spore catch was recorded on 14th August which corresponds to temperature (max. 27.5°C and min. 22.6°C), relative humidity (90%), rainfall (4.9 mm) and wind speed (2.9 km/hr) whereas during 2008, the highest (2.33%) spore catch was recorded on 31st October which corresponds to temperature (max. 27.4°C and min. 16.1°C), relative humidity (93.5%), rainfall (nil) and wind speed (1.5 km/hr). It was evident from the present finding that a good moisture content with high relative humidity (> 90%) and moderate temperature (27 – 28°C) had influence on the concentration of airspora in addition ot the available sources (substrates) within the indoor air of libraries. The finding was in agreement with the findings of other workers (Vittal and Glory, 1985; Upadhyaya and Jain, 2005).

The average monthly contributions varied quantitatively in both the years. Table 2 and Fig. 1 revealed monthwise concentration of spores. During 2007, August registered highest with 6.42% whereas during 2008, October registered highest with 7.66%. Highest concentration of spores in these months might be due to the existence of favourable conditions such as high relative humidity (> 80%), moderate temperature (29°C) and rainfall (2 – 3 mm). Similar view was opined by other workers (Singh, *et. al.*, 1990; Majumdar and Hazara, 2005).

In the present study, the binding materials, fragments and pieces of damp deteriorated papers, patches of exudate and excreta of insects, lower zoonatics and birds, dust, etc. contributed a number of spores by allowing as substrate for harbor. Disturbances caused by physical, mechanical and human activity might induce the movement of the harboring substrates, consequently initiate take off of the spores and diffuse in the air. The present finding substantiate to the previous results reported as preliminary works (Devi and Singh, 1997a and 1997b). The existence of airborne fungal biopullutants qualitatively and quantitatively as well as fluctuations in their occurrence, percentage, frequency, etc. are influenced by the substrates available within the indoor environment in addition to meteorological factors and disturbances to ambient air current inside the library room.

A large number of known biodeteriogenic and allergenic fungal spore types like *Aspergillus, Alternaria, Chaetomium, Cladosporium, Curvularia, Epicoccum, Fusarium, Cunninghamella, Rhizopus, Penicillium* etc. (Agarwal and Shivpuri, 1974) were detected from the indoor air of Standard College Library. Staff members who spend most of their time in the indoor environment of library are exposed to these spores leading to allergic responses, respiratory troubles etc. (Apetrei, *et. al.*, 2009). Cleaning of the library rooms with vacuum cleaner specially the shelves and floor, well ventilation of the rooms, installation of exhaust fan etc. can minimise the frequency of occurrence of fungal contaminants.

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EFFECT OF CEMENT DUST ON THE MORPHOLOGY AND LEVELS OF SOME METALS IN *ELEUSINE INDICA* L. GUERTN AND *SIDA ACUTA* BURN F.

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ABSTRACT

The effect of cement dust on *Eleusine indica* L. Guertn and *Sida acuta* Burn F. growing around a cement factory in Nigeria have been studied. Morphological characters and levels of some nutrient elements were examined. *E. indica* showed decrease in length and width of leaf, length of sheath, number and length of internode. There were increases in the number of spike and stomatal frequency while stomatal size reduced. *S. acuta* showed increase in length and width of leaf while the lengths of stem and root reduced. There was increase in stomatal frequency, index and size for both upper and lower surfaces while trichome frequency reduced. Levels of calcium and silicon increased while level of iron reduced. Accumulation of metals was highest in the leaves than in the stems and roots in both locations for most of the samples. Cement dust led to reduced growth.

Key words : Cement dust , Cement factory , *Eleusine indica* L. Guertn and *Sida acuta* Burn F., Morphological characters.

INTRODUCTION

Preliminary studies on the effect of cement dust pollution on plants in Nigeria were carried out. The main components of cement product are the oxides of calcium, iron, aluminium, silicon, sodium, potassium and sulphur (Lea, 1970; Czernin, 1962). Cement industries pollute the environment in form of dust in the surroundings of cement factories as their products escape during factory processing (Treshow, 1970; Uma *et al*, 1994). This leads to pollution of air, water and land in such environments. Cement dust has been reported to be harmful to plants in some other parts of the world. The dust forms thick crust, which interfere with light absorption, leads to stunted growth and fewer leaves in plants (Darley, 1966; Borka, 1986; Pawer *et al*, 1982; Prasad *et al*, 1991; Singh and Rao, 1981).

Plants absorb non-essential elements along with essential ones. Either essential or not, all metals become toxic at various levels of concentration within the plant (Alloway, 1990; Epstein, 1972 and Treshow, 1970).

Plants that accumulate metals at high concentrations are useful indicators for the presence of such metals in an area, and also in comparing the levels of concentrations in different places and different seasons of the year (Peterson, 1983 and Nuorteva *et al*, 1986).

The aim of this study was to examine the effect of cement dust pollution on length and level of accumulation of some metals in plants.

MATERIALS AND METHOD

The study was carried out in the surroundings of West African Portland Cement Company, Ewekoro, Ogun State.

The study locations were about 250 meters away from the factory. The control location was a similar vegetation which was some kilometers away from the polluted environment. Two plants were selected for assessment; a monocotyledon and a dicotyledon, and they were collected at fruiting stages. The plants were carefully uprooted to avoid damage of organs especially the roots.

Macro-morphological features – for each plant, ten individuals were assessed at each study location. The lengths of shoot and root were measured. Fifty matured leaves were selected at random for each plant and the length and width were measured.

Leaf Epidermal features – samples (1 x 1cm^2) for study were taken from the middle area of the leaf between the base and apex of the lamina. They were soaked in concentrated nitric acid. The epidermis were separated with needle and fine forceps, rinsed in two changes of water and transferred to 10% aqueous solution of safranin O for 3 minutes, washed in water to remove excess stain and mounted in glycerin (Ogundipe, 1992). Various epidermal characters were assessed.

Metal Analysis – Plant samples were rinsed with water and air dried. They were further dried at 60°C for 48hours. Levels of three metals, calcium, silicon and iron were examined. Analysis in the leaves stems and roots were done separately for each plant. For each organ, 0.5g was digested in 10ml of concentrated nitric acid and perchloric acid (ratio 1:1). The digested samples were standardized to 50ml by adding distilled water and were analyzed using atomic absorption spectrophotometer (Jones, 1984)

EFFECT OF CEMENT DUST ON THE MORPHOLOGY AND LEVELS OF SOME METALS IN *ELEUSINE INDICA* L. GUERTN AND *SIDA ACUTA* BURN F.

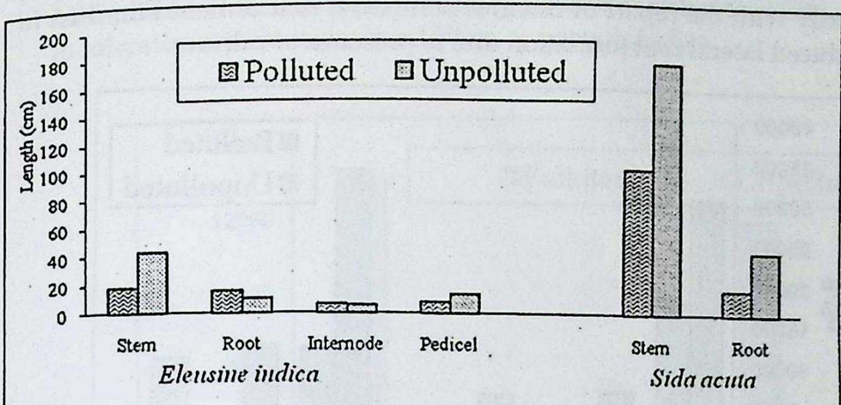

Figure 1: Effect of cement dust on stems and roots

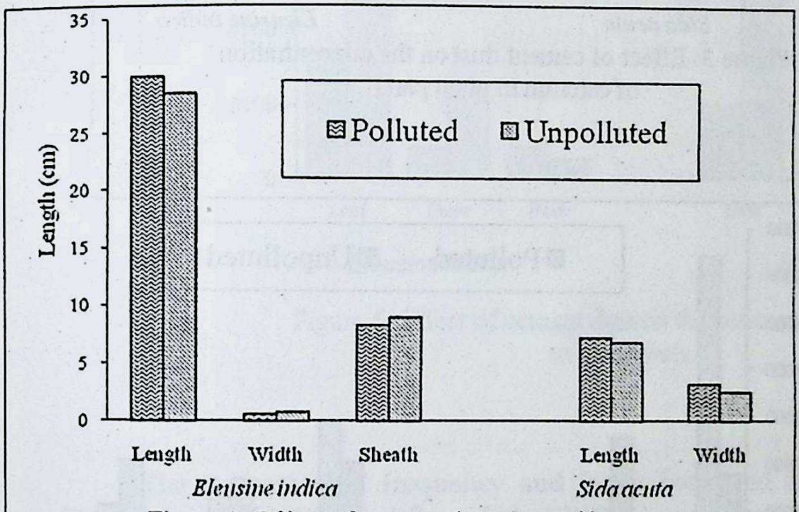

Figure 2: Effect of cement dust sizes of leaves

RESULTS AND DISCUSSION

The heights of both plants (figure 1) and the size of leaves of *E. indica* (fig. 2) are lower at the polluted area, these show that cement pollution leads to reduced growth of plants. Pawer *et al* (1982) and Prasad *et al* (1991) have reported that cement clean dust reduced total chlorophyll content, especially chlorophyll-a. Borka (1980) stated that higher cement kiln dust pollution level considerably decreases the growth and metabolic activities like photosynthesis and respiration. Also Prasad *et al* (1991) attributed the reduction in growth to the reduced intensity of light energy available for photosynthesis through covering of leaves. However, the root of *E. indica* increased in length at the polluted area, this agrees partly with the report of Emanuelson (1984) that cement kiln dust in mild doses induced lateral root formation due to presence of calcium.

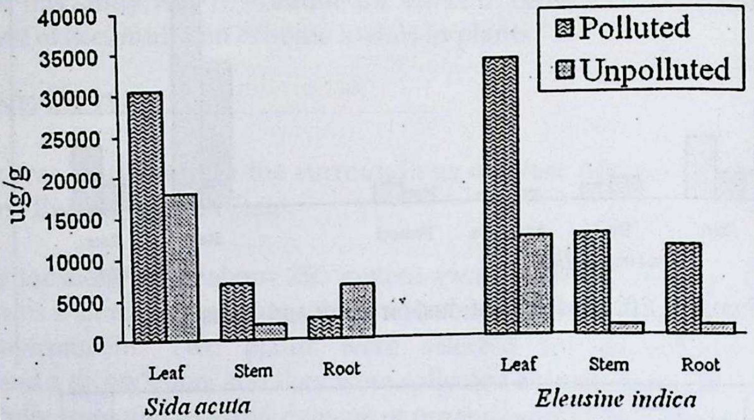

Figure 3: Effect of cement dust on the concentration of calcium in plant parts

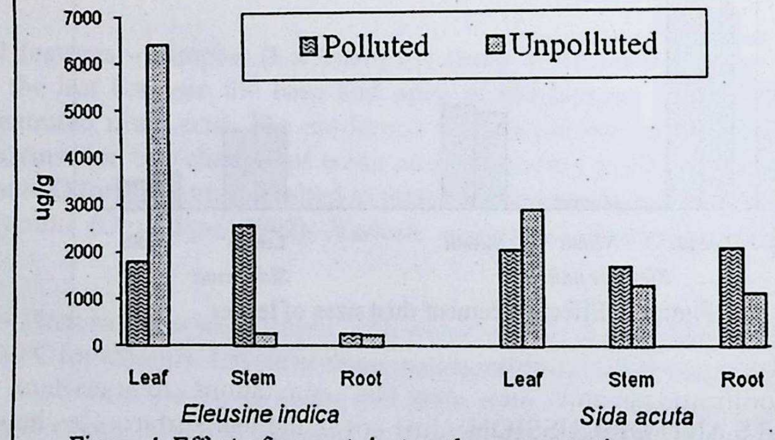

Figure 4: Effect of cement dust on the concentration of silicon in plant parts

The number of spikes and ratio of pedicel to stem in *E. indica* increased (table 1), thus cement pollution did not reduce reproductive growth, this is contrary to the report of Prasad *et al* (1991) as discovered in *Cajanus cajan* (L.)

Millsp. Such differences in effect may be due to different ability of various plants to tolerate or resist stress in addition to growth factors.

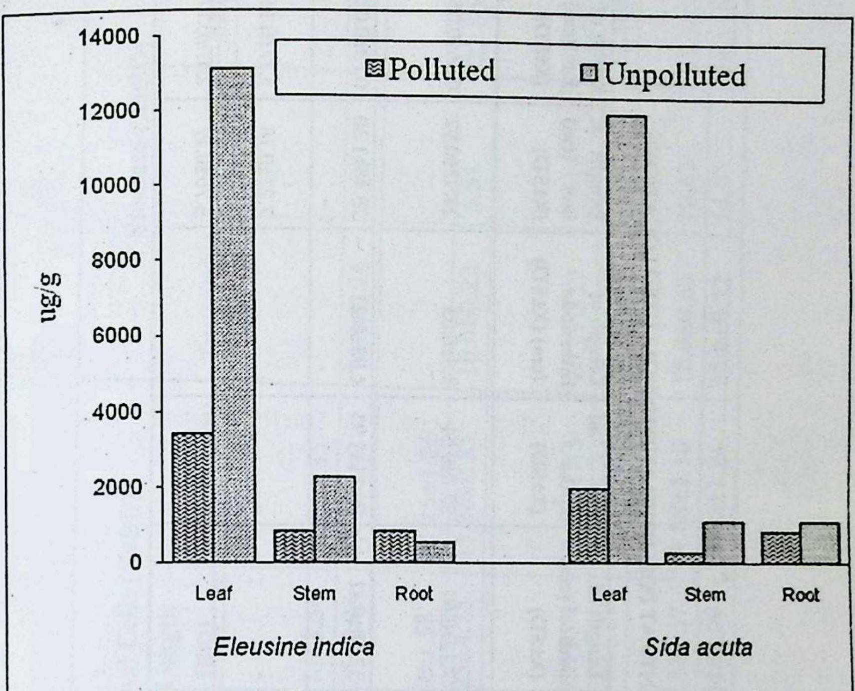

Figure 5: Effect of cement dust on the concentration of iron in plant parts

The stomata size, frequency and index increased in *S. acuta* (table 2). Chaudhary *et al* (1991) and Prasad *et al* (1991) have a similar report for stomata index but with reduced stomata frequency. These results indicate morphological modifications and they may be adaptations for gaseous exchange and transpiration when cement dust cover the leaf surface. On the other hand, the increased stomata size may be dangerous to the plant because it may encourage the penetration of cement particles into the leaf tissue as recorded by Borka (1986). These changes in characters may have taxonomic points of interest as found in *Ameria* species (Peterson, 1983).

TABLE 1: MORPHOLOGICAL CHARACTERS OF PLANTS AT POLLUTED AND UNPOLLUTED LOCATIONS

Species	Length of stem(cm) (X±SD)	Length of root (cm) (X±SD)	No of Internode (X±SD)	Length of pedicel (cm) (X±SD)	No of spike (X±SD)	Length of Internode (cm) (X±SD)	Length of laef (cm) (X±SD)	Width of Laef(cm) (X±SD)	Length of sheath (cm) (X±SD)
Eleusine indica P	17.29±1.08	14.94±1.45	2.5±0.15	7.12±0.60	27.2±0.54	5.6±0.03	24.93±0.92	0.56±0.06	8.23±0.38
C	42.25±1.68	9.89±0.66	3.5±0.16	12.58±0.41	24.11±1.93	4.83±0.22	28.48±1.30	0.73±0.02	8.93±0.22
Sida acuta P	101.50±3.88	16.5±3.22	-	-	-	-	7.21±0.18	3.17±0.11	-
C	177.50±3.83	43.8±3.20	-	-	-	-	6.75±0.17	2.47±0.17	-

Key

P – Polluted location
C – Control (Unpolluted) location

TABLE 2 : LEAF EPIDERMAL FEATURES OF THE PLANTS AT POLLUTED AND UNPOLLUTED LOCATIONS

Species/Surface	Epidermal Cells (X±SD)			Stomata (X±SD)		
	Frequency	Length (um)	Width (um)	Frequency	Index	Size (um)
Eleusine indica						
P						
Adaxial	153±2.66	48.75±3.56	21.0±1.85	-	-	-
Abaxial	218±3.67	85.25±2.95	9.75±0.39	16.5±0.26	7.04	15.75±3.60
C						
Adaxial	145.75±2.24	40.0±1.58	23.25±1.86	-	-	-
Abaxial	143.00±1.16	52.25±4.25	11.86±0.85	10.0±0.22	5.54	22.50±0.13
Sida acuta						
P						
Adaxial	69.80±1.60	47.75±2.41	20.88±1.15	20.1±0.57	22.35	27.30±1.44
Abaxial	74.00±1.44	41.75±1.57	24.50±0.74	33.3±0.64	31.03	20.50±0.81
C						
Adaxial	60.50±1.11	38.13±2.37	19.88±1.10	18.0±0.63	10.82	23.5±0.73
Abaxial	142.00±1.66	31.88±2.09	14.50±1.24	25.0±0.22	14.97	18.3±0.60

Key

P – Polluted location
C – Control (Unpolluted) location

The levels of metals vary greatly. Variations in the levels may be due to the various factors that affect the absorption and translocation of mineral nutrients by plants (Alloway, 1990; Epstein, 1972 and Foth, 1990). The accumulation of metals was generally highest in the leaves and lowest in the root at the polluted area. Levels of calcium and silicon increased with very few exceptions. In general, cement dust increased the levels of the examined metals. The effects of high level are of two folds; it may become toxic at excessive level and also it may lead to deficiency of competing essential elements (Epstein, 1972).

The study reveals that cement dust pollution have effects on morphology and level of heavy metals in plants growing around cement factory. In conclusion, cement dust pollution led to reduction in the length of plants.

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CONTRIBUTION TO A CHROMOSOME ATLAS OF THE NEPALESE FLORA I

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ABSTRACT

Reports of the chromosome counts for 281 taxa of the Nepalese flora within three families Caprifoliaceae, Compositae and Leguminosae are enumerated in this article. Of these, chromosome counts for 13 taxa are reported in the present investigation.

Key words: Flora, Chromosome counts, Genetic diversity.

INTRODUCTION

Nepal contains over 7, 000 flowering plants (Shrestha *et al.,* 2000) though the country occupies only 1 % of the total land area of the world (Joshi, 2001). This prestigious heritage of the floral diversity harbors an equal genetic diversity that is still to be explored for the evaluation, maintenance and management of its genetic resources.

It is estimated that 2, 50,000 of flowering plants known on earth and 75 % of them are cytologically untouched (Stace, 2000). The research in the Himalayan flora still remains little touched field of investigation (Wakabayashi, 1988; Dhar, 2002). The risk of extinction of species is expected to be about 20-25 % of all of the existing plant diversity on earth in the near future (Kumar and Subramanian 1986) due to human intervention affecting global climatic change and habitat loss. The report of the chromosome number for the Nepalese taxa in the present investigation is intended to give recognition of plant genetic diversity of the country to scientific world in national and international context.

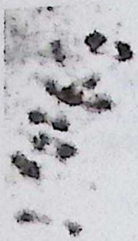
It is recorded that about 6 genera of Caprifoliaceae, 111 genera of Compositae and 80 genera of Leguminosae are present in Nepal (Hara and Williams, 1979; Press *et al.,* 2000; Rajbhandari, 2002-2003). Among them, the literature has revealed that 13 taxa for Caprifoliaceae, 41 taxa for Compositae, 227 taxa for Leguminosae are cytogenetically known. Of these, 13 taxa are reported in the present investigation.

METHODS

The chromosome counts were obtained either from microsporogenesis in flower buds or from somatic cells of root tips according to methods of Sakya and Joshi (1990) and Cota and Philbrick (1994).

RESULTS

The chromosome counts for 13 different leguminous taxa (Fig. 1-15) are reported in the present investigation. The reports of the literature review and present investigation for all the chromosome counts are listed in Table 1.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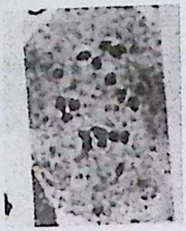
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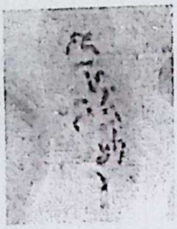
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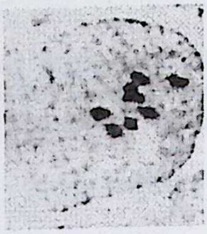
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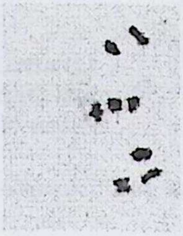
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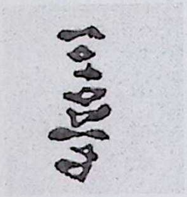
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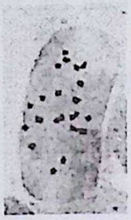
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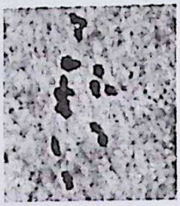
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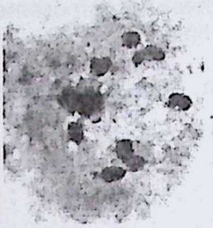
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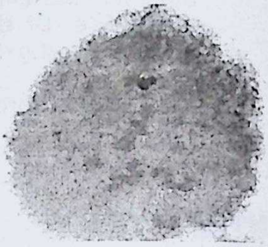
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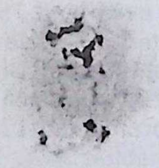
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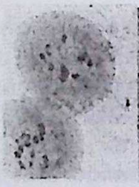
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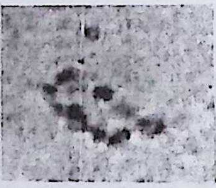
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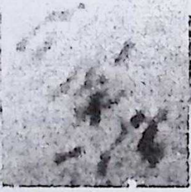
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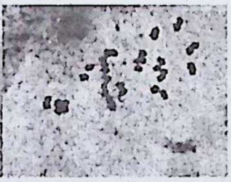
Fig. 15

Legends for Figures

Fig.1. *Alysicarpus rugosus* n=8

Fig. 2. *Aeschynomene indica* n=20

Fig. 3. *Crotalaria cytisoides* 2n=16

Fig. 4. *C. humifusa* n=8

Fig. 5. *C. pallida* n=8

Fig. 6. *C. sessiliflora* n=8

Fig..7. *Desmodium concinnum* 2n=22

Fig. 8. *D. multiflorum* n=11

Fig. 9. *D. sequax* n=11

Fig. 10..*Eriosema himalaicum* n=11

Fig.11. *E. himalaicum* (sticky metaphase I).

Fig. 12. *E. himalaicum* (cytomixis)

Fig. 13. *Parochetus communis* n=8

Fig..14. *Smithia ciliata* n=19

Fig. 15. *S. sensitiva* n=19

Table 1. Chromosome number reports for the Nepalese flora within the families Caprifoliaceae, Compositae and Leguminosae.

Caprifoliaceae

Taxon	**Chromosome number**	**Author**	**Distribution/Locality (msl)**
Leea aspera Edgew.	n=12	Malla *et al.* (1978a)	Godawari, 1375
Leycesteria formosa Wall.	n=9	Malla *et al.* (1977c)	Phulchoki, 1550.
Lonicera japonica Thumb.	n=9	Giri (1974-19750	Kirtipur, 1300
L. japonica Thunb.	2n=18	Joshi and Joshi (2001)	C, 1400
L. sympervirens Trumpet	n=18	Giri (1974-1975)	Kittiput, 1300
Sambucus adnata Wall.	2n=36	Kurosawa (1966)	Bhuspate Danra, E. Nepal, 2900msl.
S. hookeri Rehder.	2n=36	Kurosawa (1966)	GhatteKhebang, E. Nepal, 1600-2400.
Vibernum coriaceum Blume.	2n=18	Kurosawa (1966)	Ghatte Khebang, E. Nepal, 1600-2400.
Vibernum cylindricum Ham..	n=9	Malla *et al.* (1975)	Royal Botanical garden, Godawari, 1375.
V. erubescens Wall.	2n=32	Kurosawa (1966)	Mai Majuwa-Mai Pokhari, E. Nepal, 1600.
V. muliaha Hamilt.	2n=18	Kurosawa (1966)	Bhuspate Danra, E. Nepal, 2900.
V. mullaha Buch-Ham. Var. *involucrata* Buch-Ham.	n=9	Malla *et al.* (1978a)	Godawari, 1375
V. mullaha Hamilt.	n=9	Singh and Sakya (1980)	Candragiri hill, 1500

Compositae

Taxon	**Chromosome number**	**Author**	**Distribution/Locality (msl)**
Adenocoulon himalaicum Edgeworth	2n=46	Kurosawa (1971)	Phulchoki, 2800
Ageratum conyzoides L.	n=20	Maharjan (1977)	Kirtipur, 1300
A. conyzoides L.	2n=18	Karna Mallick *et al.* (2010)	Minbhawan, 1280
Ainsliaea latifolia (Don.) Schutz.	n=12	Malla *et al.* (1977b)	Phulchoki,
Anaphalis triplinereus var. *monocephala* DC. Airy Shaw	2n=14	Karna Mallick *et al.* (2010)	Phulchoki, 1900
Arctium lappa L.	2n=32, 36	Joshi and Joshi (2001)	WC 2100-3700
Artemisia indica Willd.	2n=34	Joshi and Joshi (2001)	CE 300-2400
Aster penducularis subsp. *nepalensis*	2n=18	Karna Mallick *et al.* (2010)	Kathmandu, 1300
Bidens pilosa L.	n=36	Maharjan (1977)	Kirtipur, 1300
B. pilosa var. *minor* (Blums.)	2n=36	Karna Mallick *et al.* (2010)	Kathmandu, 1300
Blumea aromatica DC.	n=18	Malla *et al.* (1977c	Gokarna., 1330
Blumea riparia (Blums.) DC.	n=9	Malla *et al.* (1977b)	Nagarjun, 1400
Blumes mollis D. Don	n=11	Maharjan (1977)	Kirtipur, 1300
Carthamus tinctorius L.	2n=34	Joshi (1988)	Seeds from Kalaiya (Narayani zone), 96

Centipida minima (L.) A. Br. & Aschers.	2n=14	Malla *et al.* (1978a)	Godawari, 1375
Cirsium arvense Mill.	2n=34	Joshi (1988)	Kirtipur, 1300
Cosmos bipinnatus	2n=24	Joshi (1988)	Wasteland of Lalitpur, 1260
Crassocephalum crepidioides Benth.	2n=20	Maharjan (1977)	Kirtipur, 1300
C. crepidioides Benth.	2n=40	Karna Mallick *et al.* (2010)	Baneswor, 1285
Eclipta prosta (L.) L.	2n=18, 22	Joshi and Joshi (2001)	WCE, 200-1200
Eupatorium adenophorum Spreng	2n=50	Karna Mallick *et al.* (2010)	Minbhawan, 1280
Galeinsuga parviflora Cav.	2n=16	Maharjan (1977)	Kirtipur, 1300
Guizotia abyssinica Cass.	2n=30	Joshi (1988)	Seeds from Dhading (Bagmati zone), 1200
Inula cappa (Buch-Ham. Ex D. Don) DC.	2n=20, 40	Joshi and Joshi (2001)	WCE 150-2500
I. racemosa Hook. f.	2n=20	Joshi and Joshi (2001)	WCE, 2500-3700
Lactuca gracilis DC.	n=7, 2n=14	Maharjan (1977)	Kirtipur, 1300
L. sagillarioides C. B. Clarke.	n=5	Malla *et al.* (1977b)	Nagarjun, 1400
Ligularia sibirica Cass.	2n=60	Malla *et al.* (1981)	Dhorpatan, 1450
Saussurea gossipiphora D. Don	n=13	Malla *et al.* (1979)	Jatapokhari, 3000
Senecio nudicaulis Ham. ex D. Don	2n=40	Malla *et al.* (1977c)	Royal Botanical garden, Godawari, 1375.
Sigesbeckia orientalis L.	2n=20, 24, 30, 60	Joshi and Joshi (2001)	WCE, 400-2700
Sonchus arvensis L.	2n=36	Joshi (1988)	Kirtipur, 1300
S. asper (L.) Hill	2n=18	Maharjan (1977)	Kirtipur, 1300
S. asper (L.) Hill	2n=18	Karna Mallick *et al.* (2010)	Kirtipur, 1330
Spilanthus calva DC.	2n=36	Karna Mallick *et al.* (2010)	Godawari, 1500
Tagetes erecta L.	2n=24, 48	Joshi and Joshi (2001)	CE, 1800-2000
T. patula L	2n=24	Karna Mallick *et al.* (2010)	Minbhawan, 1300
Taraxacum officinale F. H. Wigg.	2n=8, 16, 18, 24, 24 + 2B, 26, 27, 32, 38, 40	Joshi and Joshi (2001)	WCE
T. officinale F. H. Wigg.	2n=14	Karna Mallick *et al.* (2010)	Lukla, 2800
Xanthium strumarium L.	2n=36	Joshi (1988)	Wasteland of Lalitpur, 1260
X. strumarium L.	2n=32	Karna Mallick *et al.* (2010)	Baneswor, 1300

Leguminosae

Taxon	Chromosome number	Author	Distribution/Locality (msl)
Abrus precatorius L.	2n=22	Joshi and Joshi (2001)	WE, 300-1100
Acacia catechu (L. f.) Willd.	2n=26	Joshi and Joshi (2001)	WCE, 200-1400
A. nolotica (L.) Willd. ex Delile	2n=26, 52, 104	Joshi and Joshi (2001)	C, 150
Aeschynomene indica L	n=20, 2n=40	Tuladhar (1978)	WCE, 200-1300. Kirtipur, 1300
A. indica L.	n=20	**Present report**	Kirtipur, 1300
Albizia julibrissin Durazz. Var. *Julibrissin*	2n=26, 52	Joshi and Joshi (2001)	E, 1300-1400
A. lebbeck (L.) Benth.	2n=26	Joshi and Joshi (2001)	WCE, 250-800
Alysicarpus rugosus (Willd.) DC.	n=8, 2n=16	Manandhar (2005)	WCE 200- 1300, Kirtipur, 1300.
A, rugosus (Willd.) DC.	n=8	**Present report**	Kirtipur, 1300
A. vaginalis (L.) DC.	n=16, 2n=32	Manandhar (2005)	WCE 70-1200, Biratnagar, 72.
Arachis hypogaea L.	2n=40	Joshi and Joshi (2001)	C, 800-1000
Astragalus chlorostachys Lindl.	n=8, 2n=16	Manandhar and Sakya (2004)	WCE 2100-3700, Tadapani, way to Ghorepani 2650
A. chlorostachys Lindl.	n=8, 2n=16	Manandhar (2005)	WCE 2100-3700, Tadapani, way to Ghorepani 2650
A, khasianus Benth.	n=8	Malla *et al.* (1977)	Physic Garden, Royal Batanical Garden 1360
A. stipulatus var. *phuchokiensis* H. Ohashi	n=12, 2n=24	Manandhar and Sakya (2004)	C 2200, Phulchoki hill
A. stipulatus var. *phuchokiensis* H. Ohashi	n=12, 2n=24	Manandhar (2005)	C 2200, Phulchoki hill
Bauhinia purpuria L.	2n=26, 28	Joshi and Joshi (2001)	WCE, 300-1600
B. vahlii Wight & Arn.	2n=28	Joshi and Joshi (2001)	WCE, 200-1300
B. variegata L.	2n=28	Joshi and Joshi (2001)	WCE, 150-1900
Butea buteiformis (Voigt.) Grierson	2n=18	Joshi and Joshi (2001)	WCE, 300-2000
B. buteiformis (Voigt.) Grierson	2n=18	Manandhar and Sakya (2003b)	WCE 200-2000, Kirtipur, 1300.
B. buteiformis (Voigt.) Grierson	n=9, 2n=18	Manandhar (2005)	WCE 200-2000, Kirtipur, 1300.
B. monosperma (Lam.) Kuntze	2n=18, 18+f, 32	Joshi and Joshi (2001)	WCE, 150-1200
Caesalpinia decapetala (Roth.) Alston.	n=11	Malla *et al.* (1977c)	Phulchoki, 1500
Cajanus cajan (L.) Huth	2n=22	Fukuda (1984)	CE, 200-2000
C. cajan (L.) Huth	n=11, 2n=22	Manandhsr (2010)	CE, 200-2000, Balkhu, 1280
Caragana gerardiana Royle.	n=8	Malla *et al.* (1978b)	Jomsom, 2100

Caragana nepalensis Kitamura	n=8	Malla *et al.* (1979)	Langtang, 3400
Cassia fistula L.	2n=24, 26, 28	Joshi and Joshi (2001)	WCE, 150-1400
C. floribunda Cav.	n=7	Malakar (1978)	WCE, 700-2200
C. floribunda Cav.	2n=14-26	Chaulagain (1997)	WCE, 700-2200 Sallaghari Bhaktapur, 1100
C. floribunda Cav.	2n=14-26	Chaulagain & Sakya (2002)	WCE, 700-2200 Sallaghari, Bhaktapur 1100
C. laevigata Willd.	n=14, 2n=28	Tuladhar (1978)	Kirtipur, 1300 •
C. mimosoides L.	2n=18, 14-30	Chaulagain (1997)	WCE, 700-2500 Taplejung, 1500
C. mimosoides L.	2n=18, 14-30	Chaulagain & Sakya (2002)	WCE, 700-2500 Taplejung , 1500
C. nairobensis Hort.	2n=20, 16-28	Chaulagain (1997)	Kirtipur, 1310
C. nairobensis Hort.	2n=20, 16-28	Chaulagain & Sakya (2002)	Kirtipur, 1310
C. occidentalis L.	n=14, 2n=28	Tuladhar (1978)	WCE, 200-1400, Swoyambhu, 1300.
C. occidentalis L.	2n=26, 28, 16-38	Chaulagain (1997)	WCE, 200-2500 Panchkhal, Kabhre 740
C. occidentalis L.	2n=26, 28, 16-38	Chaulagain (1997)	Swoyambhu 1330
C. occidentalis L.	2n=26, 28, 16-38	Chaulagain (1997)	Santapur, Routahat 114
C. occidentalis L.	2n=26, 28, 16-38	Chaulagain & Sakya (2002)	Panchkhal, Kabhre 740
C. tora L.	2n=22, 16-38	Chaulagain (1997)	WCE, 450-1300 Panchkhal, Kabhre 740
C. tora L.	2n=22, 16-38	Chaulagain (1997)	Swoyambhu 1330
C. tora L.	2n=22, 16-38	Chaulagain (1997)	Santapur, Routahat 114
C. tora L.	2n=24, 26, 28 52	Joshi and Joshi (2001)	WCE, 450-1300
C. tora L.	2n=22, 16-38	Chaulagain & Sakya (2002)	WCE, 450-1300 Panchkhal, Kabhre 740
Cicer arietinum L.	n=8	Kumar (1976)	WCE, 150-1300. Botanical Garden (CDB), Kirtipur, 1300
Cicer arietinum L.	2n=16	Fukuda (1984)	Kathmandu market, 1250
C. cabulicum L.	n=8	Kumar (1976)	Botanical Garden (CDB), Kirtipur, 1300
Crotalaria albida Heyne ex Roth.	n=8, 2n=16	Bajracharya (1978)	WCE, 450-2200 Pharping, 1340
C. albida Heyne ex Roth.	2n=16	Bajracharya and Joshi (1979)	WCE, 450-2200 Pharping, 1340
C. albida Heyne ex Roth.	n=8	Bajracharya and Joshi (1980)	WCE, 450-2200 Pharping, 1340
C. cytisoides Roxb. Ex DC.	2n=16	Manandhar and Sakya (2003b)	WCE, 1200-1900, Nagarjun hill, 1520
C. cytisoides Roxb. ex DC.	n=8, 2n=16	Manandhar (2005)	WCE, 1200-1900, Manichur forest, 1550
C. cytisoides Roxb. ex DC.	2n=16	**Present report**	Kuleswor, 1250
C. humifusa Graham ex Benth.	n=8, 2n=16	Bajracharya (1978)	WCE, 1800-1900 Kirtipur, 1300

C. humifusa Graham ex Benth	2n=16	Bajracharya and Joshi (1979)	WCE, 1800-1900 Kirtipur, 1300
C. humifusa Graham ex Benth	n=8	Bajracharya and Joshi (1980)	WCE, 1800-1900 Kirtipur, 1300
C. humifusa Graham ex Benth	n=8	**Present report**	Chovar, 1350
C. juncea L.	n=8, 2n=16	Bajracharya (1978)	Kirtipur, 1330
C. juncea L	2n=16	Bajracharya and Joshi (1979)	Kirtipur, 1330
C. juncea L	n=8	Bajracharya and Joshi (1980)	Kirtipur, 1330
C. pallida Aiton.	n=8, 2n=16	Bajracharya (1978)	CE, 200-1370 Ilam, 1250
C. pallida Aiton.	2n=16	Bajracharya and Joshi (1979)	CE, 200-1370 Ilam, 1250
C. pallida Aiton.	n=8	Bajracharya and Joshi (1980)	CE, 200-1370 Ilam, 1250
C. pallida Aiton.	n=8, 2n=16	Manandhar (2005)	CE, 200-1370, Bot. garden (CDB), Kirtipur, 1300
C. pallida. Aiton.	n=8	**Present report**	Kirtipur, 1300
C. sericea Retz.	n=8, 2n=16	Bajracharya (1978)	Dang, 800
C. sericea Retz.	2n=16	Bajracharya and Joshi (1979)	Dang, 800
C. sericea Retz.	n=8	Bajracharya and Joshi (1980)	Dang, 800
C. sessiliflora L.	n=8, 2n=16	Bajracharya (1978)	Kirtipur, 1300.
C. sessiliflora L.	2n=16	Bajracharya and Joshi (1979)	Kirtipur, 1300.
C. sessiliflora L.	n=8	Bajracharya and Joshi (1980)	Kirtipur, 1300.
C. sessiliflora L.	n=8, 2n=16	Manandhar (2005)	WCE, 200-2800 Bajrajogini forest, 1520
C. sessiliflora L.	n=8	**Present report**	Kuleswor,.1250
Desmodium concinnum DC.	n=11, 2n=22	Ranjit (1978)	Pharping, 1340
D. concinnum. DC.	2n=22	**Present report**	Kuleswor, 1250
D. concinnum var. *concinnum* DC.	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Palung, 2050
D. concinnum var. *concinnum* DC.	n=11, 2n=22	Manandhar (2005)	Palung, 2050
D. concinnum var. *retusum* (D. Don) H. Ohashi	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Nagarjun forest, 1600
D. concinnum var. *retusum* (D. Don) H. Ohashi	2n=22	Manandhar and Sakya (2003b)	Nagarjun forest, 1600
D. concinnum var. *retusum* (D. Don) H. Ohashi	n=11, 2n=22	Manandhar (2005)	Nagarjun forest, 1600
D. confertum DC.	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Dakhinkali forest, 1550
D. confertum DC.	n=11, 2n=22	Manandhar (2005)	Dakhinkali forest, 1550
D. elegans DC. Ssp. *elegans* var. *elegans*	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Shivapuri, 2000
D. elegans DC. Ssp. *elegans* var. *elgans*	n=11, 2n=22	Manandhar (2005)	Phulchoki, 2200

D. elegans ssp. *elegans* var. *nutans* (Hook.) H. Ohashi	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Kharidhunga, 2400
D. elegans ssp. *elegans* var. *nutans* (Hook.) H. Ohashi	n=11, 2n=22	Manandhar (2005)	Kharidhunga, 2400
D. floribundum DC.	n=11, 2n=22	Ranjit (1978)	Pharping, 1340
D. gangeticum (L.) DC.	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Kapilbastu, 150
D. gangeticum (L.) DC.	2n=22	Manandhar (2005)	Kapilbastu, 150
D. gyrans DC.	n=11, 2n=22	Ranjit (1978)	Pharping, 1340
D. heterocarpon (L.) DC.	n=11, 2n=22	Ranjit (1978)	Pharping, 1340
D. heterocarpon (L.) DC. var. *heterocarpon*	n=11, 2n=22	Manandhar and Sakya (2003a)	Namobuddha hill, 1550
D. heterocarpon (L.) DC. var. *heterocarpon*	2n=22	Manandhar and Sakya (2003b)	Namobuddha, 1550
D. heterocarpon (L.) DC. var. *heterocarpon*	n=11, 2n=22	Manandhar (2005)	Namobuddha, 1550
D. heterocarpon (L.) DC. var. *heterocarpon*	n=11, 2n=22	Manandhar and Sakya (2009^{a})	Namobuddha, 1550
D. heterocarpon var. *strigosum* Meeunwen	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Gokarna forest, 1330
D. heterocarpon var. *strigosum* Meeunwen	n=11, 2n=22	Manandhar (2005)	Gokarna forest, 1330
D. heterocarpon var. *strigosum* Meeunwen	n=11, 2n=22	Manandhar and Sakya (2009^{a})	Gokarna, 1330
D. heterophyllum (Willd.) DC.	2n=22	Manandhar and Sakya (2003^{a})	E 200
D. laxiflorum DC.	n=11, 2n=22	Manandhar and Sakya (2003^{a})	Nagarjun forest, 1500
D. laxiflorum DC.	2n=22	Manandhar and Sakya (2003b)	Nagarjun forest ,1500
D. laxiflorum DC.	n=11, 2n=22	Manandhar (2005)	Nagarjun forest,1500
D. microphyllum forma *glabrum* H. Ohashi	2n=22	Manandhar and Sakya (2003^{a})	E, 1450-2500
D. microphyllum (Thunb.) DC.	2n=22	Manandhar and Sakya (2003b)	Panouti roadside, 1420
D. microphyllum (Thunb.) DC.	n=11, 2n=22	Ranjit (1978)	Pharping jungle, 1340
D. microphyllum var. *macrocarpum* Schindl.	n=11, 2n=22	Manandhar and Sakya (2003^{a})	CE, Kirtipur, 1320
D. microphyllum var. *microphyllum* (Thunb.) DC.	n=11, 2n=22	Manandhar and Sakya (2003^{a})	WCE, 1800-2300 Panouti, 1420
D. multiflorum DC.	n=11, 2n=22	Manandhar and Sakya (2003a)	WCE 1800-2600 Nagarjun, 1510
D. multiflorum DC.	n=11, 2n=22	Manandhar (2005)	WCE 1800-2600 Nagarjun, 1510
D. multiflorum DC.	n=11	**Present report**	Kuleswor, 1230
D. oojenense (Roxb.) H. Ohashi	n=11, 2n=22	Manandhar and Sakya (2003a)	C, 1200-1300
D. podocarpum subsp. *oxyphyllum* var. *mandschuricum* Maxim.	n=11, 2n=22	Manandhar and Sakya (2003a)	CE, 1200-1700 Namobuddha, 1660
D. podocarpum subsp. *Oxyphyllum* var. *Mandschuricum* Maxim.	2n=22	Manandhar and Sakya (2003b)	CE, 1200-1700 Namobuddha, 1660

D. podocarpum subsp. *oxyphyllum* var.*mandschuricum* Maxim.	n=11, 2n=22	Manandhar (2005)	CE, 1200-1700 Namobuddha, 1660
D. podocarpum subsp. *oxyphyllum* (DC.) H. Ohashi var. *oxyphyllum*	n=11, 2n=22	Manandhar and Sakya (2003a)	CE, 1300-2300 Phulchoki, 2200
D. podocarpum subsp. *oxyphyllum* (DC.) H. Ohashi var. *oxyphyllum*	n=11, 2n=22	Manandhar (2005)	CE. 1300-2300 Nagarjun forest, 1500
D. podocarpum DC. subsp. *podocarpum*	n=11, 2n=22	Manandhar and Sakya (2003a)	WCE, 1600-2000 Namobuddha, 1780
D. podocarpum DC. subsp. *podocarpum*	2n=22	Manandhar and Sakya (2003b)	WCE, 1600-2000 Namobuddha, 1780
D. podocarpum DC. Subsp. *podocarpum*	n=11, 2n=22	Manandhar (2005)	WCE, 1600-2000 Namobuddha, 1780
D. sequax Wall.	n=11, 2n=22	Manandhar and Sakya (2003a)	WCE, 800-2200 Phulchoki forest, 2150
D. sequax Wall.	n=11, 2n=22	Manandhar (2005)	WCE, 800-2200 Phulchoki forest, 2150
D. sequax Wall.	n=11	**Present report**	Kuleswor, 1230
D. triflorum (L.) DC.	n=11, 2n=22	Ranjit (1978)	WCE, 600-2300 Kirtipur, 1300
D. triflorum (L.) DC.	n=11, 2n=22	Manandhar and Sakya (2003a)	WCE, 600-2300 Dhulikhel, 1420
D. triflorum (L.) DC.	2n=22	Manandhar and Sakya (2003b)	WCE, 600-2300 Dhulikhel, 1420
D. triflorum (L.) DC.	n=11, 2n=22	Manandhar (2005)	WCE, 600-2300 Dhulikhel, 1420
D. velutinum var. .*sikkimense* H. Ohashi	n=11, 2n=22	Manandhar and Sakya (2003a)	C, Nepal Himalaya
D. velutinum (Willd.) DC. var. *velutinum*	2n=22	Manandhar and Sakya (2003a)	E, 300-1500
Dolicos lablab L.	n=11, 2n=22	Sharma (1976)	Kathmandu market, 1250
Entada phaseoloides (L.) Merr.	2n=28	Joshi and Joshi (2001)	E, 350-1600
Eriosema himalaicum H. Ohashi	n=11, 2n=22	Manandhar (2005)	WCE, 900-3000 Namobuddha, 1550
E. himalaicum H. Ohashi	n=11, 2n=22	Manandhar and Sakya (2008)	WCE, 900-3000 Namobuddha, 1550
E. himalaicum H. Ohashi.	n=11	**Present report**	Namobuddha, 1550
Erythrina herbacea L.	n=12	Sharma (1976)	Kirtipur, 1300
E. indica Lam.	n=12	Sharma (1976)	Kirtipur, 1300
Flemengia strobilifera (L.) W. T. Aiton	2n=22	Joshi and Joshi (2001)	WCE, 300-2300
Glycine max (L.) Merr.	n=12, 2n=40	Sharma (1976)	CE, 1000-1700
G. max (L.) Merr.	2n=40	Fukuda (1984)	CE, 1000-1700
I. arrecta Hochst ex A. Rich	2n=16	Thapa (1995)	C, 1200-

I. astragalina DC.	2n=16	Thapa (1995)	C, 1600-
Indigofera bracteata Graham ex Baker var. *khasiana* Sanjappa	n=8, 2n=16	Manandhar (2005)	C, Simbhanjyang, 2400
I. cassoides Rottl ex DC.	2n=16	Thapa (1995)	WCE, 210-3100
I. cassoides Rottl ex DC.	n=8, 2n=16	Manandhar (2010)	WCE, 210-3100, Kirtipur 1280
I. dosua Buch-Ham.ex D. Don	2n=48	Thapa (1948)	WCE, 600-3600
I. gerardiana Wall. ex Baker var. *heterantha* (Wall. ex Brandis) Baker Hook. f.	2n=48	Thapa (1995)	WCE, 600-1500
I. heterantha Wall. ex Brandis	2n=48	Thapa (1995)	WCE, 600-3500
I. linifolia (L. f.) Retz.	2n=16	Thapa (1995)	WCE, 100-1300
I. trifoliata var. *nepalensis* Ohashi	2n=16	Thapa (1995)	E, 1200
I. trifoliata L. var. *trifoliata*	2n=16	Thapa (1995)	C, 800-2400
I. trifoliata L. var. *trifoliata*	n=8, 2n=16	Manandhar (2005)	Kirtipur, 1300
Lathyrus aphaca L.	n=7, 2n=14	Kumar (1976)	Thimi, Bhaktapur, 1300
L. odoratus L.	n=7, 2n=14, 28	Kumar (1976)	Botanical Garden (CDB), Kirtipur, 1300
L. sativus L.	n=7, 2n=14	Kumar (1976)	Cultivated fields, Birganj, 90
L. sativus L.	n=7, 2n=14	Kumar (1976)	Cultivated fields, Biratnagar, 76
Lespideza eriocarpa DC.	n=9, 2n=18	Manandhar (2005)	C, Palung road side 2050
L. gerardiana Graham	n=9, 2n=18	Manandhar (2005)	WC, 2400-2600 Namobuddha hill, 1550
L. juncea var. *sericea* (Thunb.) F. B. Forbes	n=10, 2n=20	Manandhar (2005)	WC, 1900-2000 Namobuddha hill top, 1650
Lens culinaris Medik.	2n=14	Fukuda (1984)	WC, 150-1000 Kathmandu market, 1250
Lens culinaris Medik.	2n=14	Pant (1992)	WC, 150-1000 Kathmandu market, 1250
L. esculanta Moench.	n=7, 2n=14, 28	Kumar (1976)	Cultivated field, Kirtipur, 1300
Lotus corniculatus L.	n=6	Singh and Sakya (1980)	WC, 3000-3700 Chandragiri hill, 1470
Macrotyloma uniflorum (Lam.) Verdc.	2n=20, 22, 24	Joshi and Joshi (2001)	CE, 450-2800
Medicago denticulata Willd.	n=8, 2n=16	Manandhar (2005)	WCE, 200- 1400 Janakpur, 660

M. lupulina L.	2n=16	Dhakhwa (1979)	WC 500-4000
M. lupulina L.	n=8, 2n=16	Manandhar (2005)	WC 500-4000 Janakimandir, 660
Mellilotus alba Medic. ex Desr.	2n=16	Dhakhwa (1979)	WE, 200-1000
M. indica (L) All.	n=12, 2n=24	Manandhar (2005)	CE, 500-3600 Janakpurdham, 660
Mimosa pudica L.	2n=48, 52	Joshi and Joshi (2001)	CE, 200-1200
Moghania strobilifera (L.) O. Kuntze.	n=11	Malla *et al.* (1975)	Royal Botanical garden,Godawari, 1275.
Parochetus communis Buch-Ham.	2n=16	Dhakhwa (1979)	WCE, 900-4000
P. communis Buch-Ham.	n=8	Malakar (1978)	WCE, 900-4000
P. communis Buch-Ham.	n=8, 2n=16	Manandhar (2005)	WCE, 900-4000 Kharidhunga, 2400
P. communis Buch-Ham.	n=8	**Present report**	Kuleswor, 1230
Phaseolus angularis Willd.	2n=22	Shrestha (1982)	Kathmandu market, 1250
P. aureus Roxb.	n=11	Sharma (1976)	Kathmandu market, 1250
P. aureus Roxb.	2n=22	Shrestha (1982)	Kathmandu market, 1250
P. calcaratus Roxb	n=12, 2n=24	Sharma (1976)	Kathmandu market, 1250
P. calcaratus Roxb	2n=22	Shrestha (1982)	Kathmandu market, 1250
P. multiflorus Willd.	2n=20	Shrestha (1982)	Kathmandu market, 1250
P. mungo L.	2n=22	Shrestha (1982)	Kathmandu market, 1250
P. vulgaris L.	2n=22	Sharma (1976)	Kathmandu market, 1250
P. vulgaris L.	2n=16, 18, 22	Jha (1979)	Kathmandu market, 1250
P. vulgaris L.	2n=22	Shrestha (1982)	Kathmandu market, 1250
Piptanthus nepalensis (Hook) D. Don	n=9	Malakar (1978)	WCE, 2000-3800
P. nepalensis (Hook) D. Don	2n=18, 27	Manandhar and Sakya (2003b)	WCE, 2000-3800 Ghorepani, 2750
P. nepalensis (Hook) D. Don	n=9, 2n=18, 27	Manandhar (2005)	WCE, 2000-3800 Kalinchok, 3300
P. nepalensis (Hook) D. Don	n=9, 2n=18, 27	Manandhar (2005)	WCE, 2000-3800 Chandanbari, 3400
P. nepalensis (Hook) D. Don	n=9, 2n=18	Manandhar (2005)	WCE, 2000-3800 Ghorepani, 2750
Psophocarpus tetragonolobus (L.) DC	2n=18	Shrestha (1982)	Kathmandu market, 1250
Pisum arvense L.	n=7, 2n=14	Kumar (1976)	Botanical Garden (CDB), Kirtipur 1300
P. arvense L.	2n=14	Shrestha (1979)	Kirtipur, 1300
P. sativum L.	n=7, 2n=14	Kumar (1976)	WCE, 1200-4000 Botanical Garden (CDB), Kirtipur, 1300
P. sativum L.	2n=14	Shrestha (1979)	Kirtipur, 1300
Proralea corylifolia L.	n=10	Malla *et al.* (1977b)	Godawari, 1375

Pueraria peduncularis (Benth.) Graham ex Benth.	n=11	Malla *et al.* (1977c)	Gosainkunda, 36000
P. tuberosa (Roxb. ex Willd.) DC.	2n=22	Joshi and Joshi (2001)	WC, 300-1500
Saraca asoca (Roxb.) W. J. de Wilde	2n=24	Joshi and . Joshi (2001)	CE, 150-1400
Shuteria hirsuta Baker	n=11, 2n=22	Manandhar (2005)	CE, 1200-1800 Shivapuri, 1800
Shuteria involucrata var. *glabrata* (Wight & Arn.) H. Ohashi.	n=11, 2n=22	Manandhar (2010)	CE, 1200-1800 Shivapuri, 1800
Smithia ciliata Royle	n=11, 2n=22	Tuladhar (1978)	WCE, 1200-2800 Kirtipur, 1300
S. ciliata Royle	n=19, 2n=38	Manandhar (2005)	WCE, 1200-2800 Panighat, 1700
S. ciliata Royle	n=19, 2n=38	Manandhar (2009b)	WCE, 1200 2800 Panighat, 1700
S. ciliata Royle	n=19	**Present report**	Kuleswor home gerden, 1250
S. sensitiva Ait.	n=19, 2n=38	Tuladhar (1978)	Kirtipur, 1300
S. sensitiva Ait.	n=19	Malakar (1978)	Kirtipur, 1300
S. sensitiva Ait.	n=19, n=38	Manandhar (2010)	Kirtipur, 1310
S. sensitiva Ait.	n=19	**Present report**	Kirtipur, 1300
Trifolium repens L.	2n=32	Dhakhwa (1979)	C, 1500-2500
T. repens L.	2n=32	Giri and Saran (1991)	C, 1500-2500
Trigonella emodi Benth.	n=8	Malla *et al.* (1977)	WCE, 1300-4900
T. foenum-graecum L.	2n=16	Dhakhwa (1979)	Kathmandu market, 1250
Uraria picta (Jacq.) Desv. ex DC.	2n=16, 22	Joshi and Joshi (2001)	W, 1000-1100
Vicia angustifolia L.	2n=12	Malla *et al.* (1981)	WCE, 200-4000
V. faba L.	n=6, 2n=12	Kumar (1976)	Godawari, Lalitpur 1360
V. faba L.	n=6, 7	Malakar (1978)	Botanical Garden (CDB), Kirtipur, 1300
V. faba L.	2n=12	Fukuda (1984)	Kathmandu, 1250
V. hirsuta L.	n=7, 2n=14	Kumar (1976)	WCE, 200-2700
V. hirsuta L.	n=7, 2n=14	Malakar (1978)	Wheat field, Kirtipur 1300
V. sativa L.	n=6, 7, 2n=14	Malakar (1978)	Kirtipur, 1300
V. tetrasperma L.	n=7, 2n=14	Kumar (1976)	Kirtipur !300
V. tetrasperma L.	n=7	Malakar (1978)	Wheat field, Kirtipur 1300
Vigna sisensis (L.) Savi.	2n=24	Sharma (1976)	Kathmandu market, 1250
V. sisensis (L.) Savi.	2n=24	Giri (1987)	Khumaltar, Lalitpur 1250
Zornia diphyla Pers.	n=5, 2n=10	Tuladhar (1978)	Kirtipur, 1300

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CHECK LIST OF ECONOMIC PLANTS OF ROURKELA AND ADJOINING REGIONS (SUNDARGARH DISTRICT)-ODISHA

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ABSTRACT

Floristic survey was undertaken in and around Rourkela city. During the survey,One hundred one angiospermic species were collected from different locality with their local names and uses. The species were arranged according their uses, there after these are arranged alphabetically with their habit ,family name and parts used. The species were dried and deposited as herbarium specimen in P.G. Department of Botany Govt. College Rourkela. The conservation strategies are also suggested.

Key words : Floristic survey, Rourkela city, Angiospermic species .

INTRODUCTION

Rourkela , the steel city of Orissa, is located in Sundargarh district. It is situated in 21 ^{0} 36^1 to 22^{0}32^{1}north latitude and 83^0 32^1 to 85^{0}22^{1}east latitude, at the elevation of 219 metres above mean sea level. it is very rich in mineral resources like coal mines, lime stones and dolomite. It had rich vegetation before the establishment of steel plant. But due to establishment of steel plant, the natural flora have degraded and many species have been extinct. This district is dominated with tribal population . The climate is tropical monsoon, hot and dry summer having high humidity. Rainfall is due to south east monsoon. i.e. in the month of June-July. There is no inventory of natural flora although these areas are rich in flora and vegetation. Haines (1921-25) did not botanize these regions. Subsequent worker,Mooney (1950) also did not give due attention to survey these areas. Saxena and Brahmam(1994) compiled the flora of Orissa but collection of economic plant is very scanty. Realizing the importance of biodiversity, attempt have been made to explore the floristic elements of Rourkela and adjoining areas to document the information regarding the uses of plant for posterity. The survey was undertaken under the auspices of P.G. Department of Botany Govt. College, Rourkela. Information was collected from the tribal people and the species was preserved as herbarium specimen and deposited in the P.G. Department of Botany Govt. College, Rourkela . The species were grouped according to their uses. Then the species were arranged alphabetically along with their family, local name, synonyms and part used correct nomenclature was also given to each species. conservation strategies were suggested. Some sporadic reports are made by Achary *et al.(*2008) and Subudhi *et al.*,(2008).

Table 1 : List of species with their family ,local names , habit and uses.

Name of species	habit	Family	Part's Used	Local name
Cereals				
Echinochloa colonum (L.)Link	H	Poaceae	Grains	Suan
Eleusine coracana (L.) Gaertn.	H	Poaceae	Grains	Mandia
Hordeum vulgare L.	H	Poaceae	Grains	Barley
Oryza sativa L.	SH	Poaceae	Grains	Dhan
Pennisetum typhoides (Burm.f)Stapf	H	Poaceae	Grains	Bajra
Sorghum vulgare Pers.	H	Poaceae	Grains	Joar
Zea mays L.	SH	Poaceae	Grains	Maka
Spices and condiments				
Allium cepa L.	H	Liliaceae	Bulb and leaves	Piaja
Allium sativum L.	H	Liliaceae	Bulb and leaves	Rasun
Amomum aromaticum Roxb.	SH	Zingiberaceae	Seed	alaichi
Amomum subulatum Roxb.	SH	Zingiberaceae	Seed	Bada alaichi
Capsicum annum L.	H	Solanaceae	Fruits	Lanka
Capsicum frutescens L	H	Solanaceae		dhanilanka
Coriandrum sativum L.	H	Apiaceae	Seeds	Dhania
Cuminum cyminum L.	H	Apiaceae	Seeds	Jira
Curcuma amada Roxb.	H	Zingiberaceae	Rhizome	ambada
Curcuma longa L.	H	Zingiberaceae	Rhizome	Haldi
Elettaria cardamomum L.	H	Zingiberaceae	Fruits	gujrati
Foenicum vulgare Mill.	H	apiaceae	Seeds	Panmahuri
Nigella sativa L.	SH	Ranunculaceae	Seeds	Kalajira
Papaver somniferum L.	SH	papaveraceae	Seeds	Aphim
Trachyspermum ammi (L.)Spar.		apiaceae	Seed	Juani
Trigonella foenum – graceum L.	H	Fabaceae	Seed	Menthi
Zingiber officinale Rosc.	H	zingiberaceae	Rhizome	Ada
Oil yielding				
Arachis hypogea L.	H	Fabaceae	Seed	Chinabadam
Argemone mexicana L.	H	Papaveraceae	Seed	Agara
Bacopa monnieri (L.) Penn.	H	Scorphulariaceae	Leaves	Brahmi
Brassica campestris L.	H	Brassicaceae	Seed	Sorisha
Brassica juncea L.	H	Brassicaceae	Seed	Anasorisha
Carthamus tinctorius L.	H	Asteraceae	Seed	Kusum
Centella asiatica Urb.	H	Scorphulariaceae	Leaves	Thalkudi

Helianthus annus L.	SH	Asteraceae	Seed	Suryamukhi
Mentha longifolia L.	H	Lamiaceae	Leaves	Jangalipodina
Mentha spicata L.	H	Lamiaceae	Leaves	Podina
Ocimum bassilicum L.	H	Lamiaceae	Leaves	Dhala tulsi
Ocimum cannum Sims	H	Lamiaceae	Leaves	Ganga tulsi
Ocimum sanctum L.	H	Lamiaceae	Leaves	Tulsi
Papaver somniferum L.	H	Papaveraceae	Seed	Aphim
Sesamum indicum L.	H	Pedaliaceae	Seed	Rasi
		Vegetables		
Abelmoschus esculentus L.	SH	Malvaceae	Fruits	Bhendi
Alocasia indica Spach.	H	Araceae	Leavesand corm	Saru
Alternanthera sessilis L.	H	Amaranthaceae	Leaves	Madaranga
Amaranthus caudatus	H	Amaranthaceae	Leaves	Khadasag
Amaranthus oleraceus L.	H	Amaranthaceae	Leaves	lalkhada
Amaranthus spinosus L.	H	Amaranthaceae	Leaves	Kantasag
Amaranthus tricolor L.	H	Amaranthaceae	Leaves	Nautia
Amaranthus viridis L.	H	Amaranthaceae	Leaves	Jangalikhada
Ananas comosus Schult.f.	SH	Bromeliaceae	Fruits	Sapuri
Beta vulgaris L.	H	Chenopodiaceae	Leaves,root	beet
Boerhavia diffusa L.	H	Nyctaginaceae	Leaves	Puruni
Brassica oleracea L.var botrytis L.	H	Brassicaceae	Whole plant	Phulkobi
Brassica oleracea L.capitata L.	H	Brassicaceae	Whole plant	Bandhakobi
Brassica oleracea L. gongylodes L.	H	Brassicaceae	Whole plant	oalkobi
Brassica rapa L.	H	Brassicaceae	Leaves	saljam
Celosia argentea L.	H	Amaranthaceae	Leaves	nahanga
Chenopodium albumL.	H	Chenopodiaceae	Leaves	Bathuasag
Coccinia grandis (L.)Voigt	SH	Cucurbitaceae	Fruits	kunduri
Colocasia esculenta (L.)Scott.	SH	Araceae	Whole plant	saru
Commelina benghalensis L.	H	Commelinaceae	Leaves	kansiri
Corchorus capsularis L.	SH	Tiliaceae	Leaves	Nalita
Corchorus olitorius L.	SH	Tiliaceae	Leaves	pitanalita
Daucus carota L.	H	Apiaceae	Root	gajar
Glinus oppositifolius L.	H	Molluginaceae	Leaves	pitasag
Ipomoea aquatic Forsk.	SH	Convulvulaceae	Whole plant	kalam
Lycopersicon esulentum Mill.	H	Solanaceae	Fruits	Bilati baigana
Melilotus alba Desv.	H	Fabaceae	Leaves	methisag
Momordica charantia L.	SH	Cucurbitaceae	Fruits	Kalara
Musa paradisica L.	T	Musaceae	Spadix and fruits	Kanchakadali

Musa sapientum L.	T	Musaceae	Spadix and fruits	Pachilakadali
Pisum sativum L.	H	Fabaceae	Seeds	Matar
Polygonum brbatum L.	H	Polygonaceae	Leaves	Dhainsula
Polygonum plebeium L.	H	Polygonaceae	Leaves	Muthisag
Portulaca quadrifida L.	H	Portulacaceae	Leaves	Balbalusag
Raphanus sativus L.	H	Brassicaceae	Leaves	Muli
Rumex vesicarius L.	H	Polygonaceae	Leaves	khatapalanga
Spinicia oleracea L.	H	chenopodiaceae	Leaves	Palanga sag
Trigonella foenum graceium L.	H	Fabaceae	Leaves	methi
Fibre yielding				
Abelmoschus esculentus L.	SH	Malvaceae	Bark	Bhendi
Abutilon indicum L.	SH	Malvaceae	Bark	Pedipedica
Agave Americana L.	SH	Agavaceae	Leaves	Baramasi
Corchorus aestuans L.	SH	Tiliaceae	Bark	Bananalita
Corchorus capsularis L.	SH	Tiliaceae	Bark	Nalita
Corchorus olitorious L.	SH	Tiliaceae	Bark	Pitanalita
Crotolaria juncea L.	SH	Fabaceae	Bark	Chhanapata
Crotolaria prostrata Rottl.	H	Fabaceae	Bark	Bishnukarni
Linum usitatissimum L.	H	Linaceae	Bark	Atisi
Sida cordata Borss.	SH	Malvaceae	Bark	Bajramuli
Sida cordifolia L.	SH	Malvaceae	Bark	Bisiripi
Sida rhombifolia L.	SH	Malvaceae	Bark	Sahadev
Urena lobata L.	SH	Malvaceae	Bark	Jatajatia
Body and beautycare				
Aloe vera L.	H	Liliaceae	Juice of leaf	Ghikumari
Bacopa monnieri (L.) Penn.	H	Scrophulariaceae	Leaves	Brahmi
Carthamus tinctorius L.	H	Asteraceae	Leaves	Kusum
Centella asiatica Urb.	H	Apiaceae	Leaves	Thalkudi
Curcuma longa L.	SH	Zingiberaceae	Rhizome	Haldi
Cymbopogon flexusos Nees	SH	Poaceae	Leaf juice	dhanantwari
Eclipta prostrate L.	H	Asteraceae	Leaf juice	keshdura
Hedichium coronarium Koenig.	SH	Zingiberaceae	root	adaphul
Impatiens balsamina L.	H	Balsaminaceae	Leaves	haragoura
Tagetes erecta L.	H	Asteraceae	Leaves	makhamali
Tagetes patula L.	H	Asteraceae	Leaves	gendu
Wedellia chinensis L.	H	Asteraceae	Leaves	bhrungaraj

H:Herbs, SH: Shurbs,T:Trees

CONSERVATION

Due to habitat destruction, population pressure, most of the species and ecotypes have been extinct and most of them are awaiting extinction. So extensive survey should be taken up in tribal areas to collect and document the information about the plant parts and uses. There are many underutilized crop species. These species should be properly surveyed and document the information. In-situ conservation of these species should be taken on priority so that awareness should be developed among the common people about their utilitarian value and conservation will be achieved through people participation. Awareness should be developed about the underutilized plants about their utilization and conservation.

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AEROMYCOFLORA OF A CABBAGE FIELD IN IMPHAL

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ABSTRACT

Aeromycological survey of a cabbage field was carried out for two consecutive crop seasons (Nov.1999- Mar.2000 and Nov.2000- Mar.2001) in Imphal using Rotorod Air Sampler. A total of 27 fungal spore types were identified. Dominant fungal types were *Cladosporium*, Aspergilli-Penicilli, rust spores, smut spores, *Humicola*, basidiospores, ascospores, *Alternaria, Periconia, Geotricum* etc. Changes in meteorological parameters affect the occurrence and distribution of airborne fungi quantitatively.

Key words: Aeromycoflora, Cabbage, Meteorological parameters.

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important cole crops in Manipur.It is susceptible to many diseases, some of which are widespread and others are localized. The causal agents of these include bacteria, fungi, viruses, mycoplasmas and nematodes. Among the fungal diseases of cabbage Damping off (*Pythium aphanidermatum*), Dark leaf spot and blight (*Alternaria brassicicola*), White rust (*Albugo candida*), Cabbage yellows (*Fusarium oxysporum*), Downey mildew (*Peronospora parasitica*), Root rot (*Phytophthora megasperma*), Black leg (*Phoma lingam*), Powdery mildew (*Erysiphe cruciferum*) are considered most important. Various workers reported the contribution of fungal airspora to the occurrence and disease development on different crops like blackgram, rice, tomato, etc. (Berger, 1970; Singh and Devi,1997; Datar and Mayee,1982). Aerobiological study over cabbage field was carried out to detect the chief constituents of the fungal airspora based on qualitative and quantitative analysis and effect of weather parameters on the occurrence of airborne fungi.

MATERIALS AND METHODS

Air monitoring was conducted for two consecutive crop seasons (Nov.1999- Mar. 2000 and Nov. 2000- Mar. 2001) of a cabbage field in Imphal district, Manipur using Rotorod Air Sampler. In both the crop seasons (CS_1-First Crop Season and CS_2- Second Crop Season),cabbage were planted on 15th Nov. each year and harvested on 17th March of next year. Air sampling was initiated 10 days prior to the plantation of cabbage in the field and continued for 10 days after harvesting of the crop. The sampler was operated at a constant height of 1 metre above ground level for 30 minutes at 10 day intervals. Fungal spores were identified upto generic level using published literatures (Ellis,1971; Barnett and Hunter,1972; Tilak, 1989) and by comparing with reference slides. The counts were converted to concentration/m^3 of air by multiplying with conversion factor (5). The growth phases of the cabbage crop at the field were recorded regularly at 2 weekly intervals during the growing season. The average growth phases were seedling (5-20 days), early growth (rapid leaf formation) (20-60 days), early head formation (60-80 days), late head formation (100-120 days) and harvesting (130 days). During the investigation period, meteorological parameters viz. temperature, relative humidity, rainfall, wind speed etc. were obtained from ICAR Research Centre, Lamphelpat, Imphal.

Table 1 : Total number of spore catch and percentage contribution of each spore type of the total aeromycoflora over Cabbage field.

Sl. No.	Spore Type	Number of spore/m3 of air		Percentage	
		1999-2000	2000-2001	1999-2000	2000-2001
1	*Albugo*	6090	9395	2.28	2.43
2	*Cunninghamella*	750	8030	0.28	2.08
3	*Peronospora*	1470	3855	0.55	0.99
4	Ascospores	6450	8355	2.41	2.17
5	*Chaetomium*	1800	2950	0.67	0.76
6	*Erysiphe*	1010	1435	0.38	0.37
7	*Leptosphaeria*	1895	2670	0.71	0.69
8	Basidiospores	6565	10305	2.46	2.67
9	Rust spore	29100	113015	10.89	29.29
10	Smut spore	25855	30840	9.67	7.99
11	*Alternaria*	2750	3870	1.03	1.00
12	Aspergilli-Penicilli	17110	12450	6.40	3.23
13	*Bispora*	3175	2055	1.19	0.53
14	*Cercospora*	3250	1045	1.22	0.27
15	*Cladosporium*	102025	113520	38.17	29.42
16	*Curvularia*	2010	2575	0.75	0.67
17	*Drechslera*	4270	6595	1.60	1.71
18	*Geotrichum*	6420	6360	2.40	1.65
19	*Helminthosporium*	1785	2015	0.67	0.52
20	*Humicola*	20815	16210	7.79	4.20
21	*Nigrospora*	2260	2355	0.85	0.61
22	*Periconia*	6740	10450	2.52	2.71
23	*Pithomyces*	1295	1650	0.48	0.43
24	*Pseudotorula*	2415	2490	0.90	0.65
25	*Stemphylium*	900	630	0.34	0.16
26	*Tetraploa*	340	310	0.13	0.08
27	*Torula*	1695	1885	0.63	0.49
		260240	377315	97.37	97.77
	Other types	7015	8595	2.62	2.23
	GRAND TOTAL	267255	385910		

Table 2 : Correlation between monthwise average percentage contribution of fungal air-spora over a Cabbage field and the corresponding meteorological parameters

	November		December		January		February		March	
	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Spore Conc. (%)	10.76	20.35	30.46	11.41	24.02	10.19	17.92	31.68	11.68	28.57
Temp (Max) °C	24.01	23.40	21.95	23.01	27.25	24.46	22.75	22.82	25.20	28.35
Temp (Min) °C	10.30	7.85	5.73	8.99	10.64	10.32	8.64	4.91	10.31	11.6
RH (%)	66.28	60.95	80.02	71.4	57.35	75.40	78.58	80.42	64.67	48.32
RF (mm)	0.13	0.84	4.59	5.5	1.25	0.54	0.63	0.96	1.32	0.01
Wind Speed (km/hr)	2.95	3.15	3.63	4.01	5.34	3.1	2.6	2.7	3.9	6.9

RESULTS AND DISCUSSION

The components of the airspora were classified as **fungal spores** and **other types**.Analysis of spore catches revealed 27 fungal spore types. Unidentified spore types, hyphal fragments, pollen grains, insect scales etc. were grouped as "**other types**". During the present investigation, a total of 2,67,255 spores/m^3 of air were trapped in CS_1 and 3,85,910 spores/m^3 of air in CS_2 (Table 1). Fungal spores contributed 97.37% in CS_1 and 97.77% in CS_2 whereas other types contributed 2.62% in CS_1 and 2.22% in CS_2. *Cladosporium* contributed highest spore concentration (38.17% in CS_1 and 29.42% in CS_1) to the total airspora over cabbage field. Other dominant fungal types were rust spores (10.89% in CS_1 and 29.29% in CS_1) ,smut spores(9.67% in CS_1 and 7.99% in CS_1), Aspergilli-Penicilli (6.4% in CS_1 and 3.23% in CS_2),*Humicola*(7.79% in CS_1 and 4.2% in CS_2), *Periconia* (2.52% in CS_1 and 2.71% in CS_2), ascospores (2.41% in CS_1 and 2.17% in CS_2), basidiospores (2.46% in CS_1 and 2.67% in CS_2), *Alternaria* (1.03% in CS_1 and 1% in CS_2) etc. Concentration of fungal spores was highest (1.72% in CS_1 and 1.91% in CS_2) on 15th January in both the crop seasons which coincides with early growth phase of the crop. The corresponding meteorological parameters were temperature (max.)(>20°C), temperature (min.)(>4°C), and relative humidity (>80%). Fluctuations in meteorological parameters mainly relative humidity indirectly affect the concentration of spore load which is in agreement with other workers (Gregory, 1973; Shenoi and Ramalingam,1976; Hasnain,1993).

Table 2 depicted the correlation between the monthwise average percentage contribution of fungal airspora and the corresponding meteorological parameters. Concentration of fungal spores varies in different months. In CS_1 January contributed highest (30.46%) and February contributed lowest (11.41%) whereas in CS_2 January contributed highest (31.68%) and November contributed lowest (10.19%). It was evident from the present study that variation in temperature, relative humidity, rainfall, etc. affect the concentration of fungal spores in the air. The results are in corroboration with the findings of Singh and Devi,(1997) and Stepalska and Wolek,(2005).

Hyphal fragments were abundant in hot dry and windy days. Parasitic and saprophytic fungi were the main source of hyphal fragments. Dry and hot climatic condition made their contact loose with the substrate while wind current liberated them in the air. The present study would be useful in understanding the aerobiology and epidemiology of fungal diseases of cabbage in Imphal. Further, it would be very helpful to conduct such type of investigation in different agroclimatic zone of the other parts of the state.

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ALLELOPATHIC POTENTIALS OF AQUEOUS EXTRACTS FROM MAIZE (*ZEA MAYS*) ROOTS AND PIGEON PEA (*CAJANUS CAJAN* L.) LEAVES ON THE GROWTH AND GERMINATION OF SOYBEANS (*GLYCINE MAX.* L. MERRIL.)

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ABSTRACT

The aqueous extracts from maize (*Zea mays*) roots and Pigeon pea (*Cajanus cajan* L.) leaves were used to determine their allelophathic potentials on seed germination and seedling growth of soybeans (*Glycine max.* L. Merril.). The extracts brought a considerable inhibition on the germination and growth of soybeans. The degree of inhibition was directly proportional to the concentration of the extract thus suggesting that the effects of the extracts were concentration dependent. The extracts also retard the growth of radicle and plumule. The degree of retardation appeared to be more pronounced in the extracts derived from maize roots.

Key words: Allelopathy, Allelochemicals, Inhibition, *Zea mays*, *Cajanus cajan*,.

INTRODUCTION

The potentials of crop residues to exhibit allelopathy had been demonstrated widely in some developed and developing countries. For example, Chuo and Lin (1976) asserted that aqueous extracts of decomposing rice residues in soil inhibiting root growth of lettuce and rice seedlings. Also, Nak *et al* (1987) demonstrated the allelopathic potentials of stem extracts from buckwheat and perilla in barnyard grass. Similarly, Irshad and Cheema (2004) demonstrated allelopathic potentials of water extracts of rice husks, above ground foliage of barnyard grass and sorghum stalk on the germination and seedling growth of barnyard grass.

In Nigeria, allolepathic studies so far reported were concentrated on the allelopathic potentials of weeds on agricultural crops. These included the studies of Tijani- Eniola and Fawusi (1989) , Ogbe *et al* (1994) on *Chromoleana odorata* and Kayode (1998 and 2004) on *Euphorbia heterophylla* and *Aspillia africana* respectively. No studies was reported on allopathic effects of some crop residues in Nigeria until recently when Kayode and Ayeni (2009a and 2009b, Ayeni and Kayode 2009), and Ayeni *et al* (2010) worked on allelopathic effects of some crop residues on selected agricultural crops.

Maize and Pigeon pea are widely cultivated in Nigeria, Residues from these crops, like every other crops, are often left uncared for in the fields, after harvest. The same fields are often prepared almost immediately for cultivation of other crops. Thus, this study been reported here examined the allelopathic potentials of aqueous extracts from residues of maize roots and pigeon pea leaves on soybeans which constituted an important agricultural crop that is now widely cultivated in Nigeria.

Table1. Effects of aqueous extracts of maize roots on the cumulative percentage germination of soybean

Extracts g/200ml	**Soybean Germination(%) /Experimental Time (Hrs.)**		**% Inhibition**
	24	**144**	
5	0	70	27
10	0	48	50
15	0	68	29
20	0	54	44
25	0	46	52
Control	0	96	

Table 2. Effects of the aqueous extracts of Pigeon pea leaves on the cumulative percentage germination of Soybean

Extracts g/200ml	**Soybean Germination (%) / Experimental Time(Hrs.)**		**% Inhibition**
	24	**144**	
5	0	90	6
10	0	90	6
15	0	90	6
20	0	82	15
25	0	80	17
Control	0	96	

Table 3. Effects of aqueous extracts of maize roots on the radical length (cm) of Soybeans.

Extracts g/200ml	**Soybeans Germination / Experimental Time (Hrs.)**		**% Inhibition**
	24	**144**	
5	0	1.53	42.3
10	0	0.44	83.4
15	0	0.67	74.7
20	0	0.61	77.0
25	0	0.32	87.9
Control	0	2.65	

Table 4. Effects of Pigeon pea leaves on the radical length (cm) of Soybeans.

Extracts g/200ml	Soybeans Germination / Experimental Time(Hrs.)		% Inhibition
	24	144	
5	0	1.63	38.5
10	0	1.19	55.1
15	0	0.97	63.4
20	0	0.80	69.8
25	0	0.79	70.2
Control	0	2.65	

Table 5. Effects of aqueous extracts of maize roots on the plumule length (cm) of soybeans .

Extract g/200ml	Soybeans Germination /Experimental Time(Hrs.)		% Inhibition
	24	144	
5	0	0.55	61.3
10	0	0.07	95.1
15	0	0.07	95.1
20	0	0.07	95.1
25	0	0.05	96.5
Control	0	1.42	

Table 6. Effects of aqueous extracts of Pigeon pea leaves on the plumule length (cm) of soybeans.

Extracts g/200ml	Soybeans Germination / Experimental Time (Hrs.)		% Inhibition
	24	144	
5	0	0.53	62.3
10	0	0.51	64.1
15	0	0.29	79.6
20	0	0.28	80.3
25	0	0.14	90.1
Control	0	1.42	

MATERIALS AND METHODS

Soybean (*Glycine max*. (L) Merril seeds were collected from the teaching and research farm of the University of Ado Ekiti. Residues (leaves) of Pigeon pea (*Cajanus cajan* L.) were collected from a farmland in Ikere Ekiti which is about 20 kilometers from University of Ado Ekiti, Ado-Ekiti, Nigeria, after harvesting of the fruits had been carried out. Maize roots were also collected from Plant Science experimental farm after harvesting. These materials were air dried for three weeks and were later pounded using pistil and mortal.

Portions of 5g, 10g, 15g, 20g, and 25g were measured from these residues (maize roots and pigeon pea leaves). Each portion was soaked in 200ml distilled water in 500ml conical flask. The mixtures were shaken intermittently and left over overnight (24hrs.). The solution extracts were filtered and the filtrates were used afresh either as such or kept in a refrigerator for further usage. In each treatment, Petri dishes were double –layered with Whatman No.1 filter paper and five seeds of soybeans were placed in each Petri dish. The filter papers were moistened daily with five different concentrations of the extracts using syringe and needle. Treatments from each concentration were replicated ten times. Control experiment whose filter paper was moistened daily with distilled water was also set up and replicated ten times.

All the Perti dishes were put in a growth chamber at room temperature. The seeds were considered germinated upon radicle emergence. The germination of radicle and plumule growth elongation measurement was recorded at 24 hour interval. The results obtained from the extract treated seeds were compared to those obtained from the control experiment.

RESULTS AND DISCUSSION

The cumulative percentage germination for soybeans seeds in the two aqueous extracts were shown in Tables 1 and 2 respectively. The results show that both extracts brought considerable inhibitions in the germination of soybeans as fewer seeds germinated in the extract- treated seeds when compared to that of the control. The degree of inhibition was concentration dependent as the proportion of the inhibition increased with increase in the concentration of the extracts. Table 1 revealed that the aqueous maize root extract inhibited germination in the 5g, 10g, 15g, 20g and 25g extracts by 27%, 50%, 29%, 44% and 52% respectively when compared to the control. Similarly in the aqueous pigeon pea leaves extract, inhibition of germination of 6%, 6%, 6%, 15% and 17% respectively were obtained (Table 2). The effects tend to be more pronounced in the maize extract.

The effects of the aqueous water extracts from maize roots and pigeon pea leaves on the radicle lengths were shown in Tables 3 and 4 respectively. The results showed that both extracts brought about considerable retardations on the growth of the radicle lengths. In the maize extract, the radicle lengths were retarded by 42.3%, 83.4%, 74.7%, 77.0% and 87.9% in the 5g, 10g, 15g, 20g and 25g extracts-treated seeds when compared to the radicle growth obtained in the control (Table 3). Similarly in the pigeon pea extract, retardations of 38.5%, 55.1%, 63.4%, 69.8% and 76.7% respectively (Table 4) were obtained when compared to the control.

The effects of the extracts on plumule lengths were similar to those of the plumule. Table 5 revealed that the plumule lengths were retarded by 61.3%, 95.1%, 95.1%, 95.1% and 96.5% respectively in the 5g, 10g, 15g, 20g and 25g extracts-treated seeds when compared to the control while reductions of 62.3%, 64.1%, 79.6%, 80.3% and 90.1% respectively were obtained in the pigeon pea extracts (Table 6). It was also observed that the aqueous maize extracts tend to have more inhibitory effects on both the radicle and plumule. However in both extracts, the inhibitory effects increased with the increase in the concentrations of the extracts. This suggested that the effects of these aqueous extracts were concentration-dependent.

These finding supports the previous assertions of other researchers that aqueous extracts from some parts of some plants inhibit germination and growth of other plants. For example Qudia (1999) asserted that extracts form *C. gigantea* inhibited the growth of radicle and plumule lengths of *Lathyrus sativa.* Malkania (1984) also reported that extracts from the leachates of *Celtis australis* and *Juglaris regia* caused decrease in germination of certain crop plants.

Previous studies had revealed that maize roots contain saponin, an alllochemical compound, and pigeon pea contain protocatechuic, p-hydroxybenzoic, vanillic, caffeic, p-coumaric, ferulic acids (Nilufar *et al.* 2006) and cajinin (Dakora and Joseph 1986), which were identified in the bark, stem and leaves of the plant. It is suspected that they must have been responsible for the inhibition of seed germination and the retardation of seedling growths of radicle and plumule of soybeans in this study.

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EFFECT OF UREA AND SINGLE SUPER PHOSPHATE ON *IN VITRO* DECOMPOSITION OF WHEAT CROP RESIDUES BY *TRICHODERMA LIGNORUM*

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ABSTRACT

Effects of treatment with 0.21% urea , 1% single super phosphate separately and in combination on decomposition of wheat internodes, wheat leaves, wheat chaff and straw by *Trichoderma lignorum* was investigated *in vitro* for 30 days. Wheat leaves showed maximum decomposition followed by chaff, straw and wheat internodes in control as well as treated flasks. Treatment with urea as well as single super phosphate increased the rate of decomposition but it was higher in the case of urea-treated residues. However, treatment with combination of urea and single super phosphate resulted in much more increase in the rate of decomposition as compared to that with urea or single super phosphate alone. As far as separate parts are concerned, wheat internodes exhibited very good response to urea and SSP treatment followed by mixed straw.

Key words: Fungal decomposition, Wheat internodes, Wheat leaves, Wheat chaff, Wheat straw, Urea, Single super phosphate, *Trichoderma lignorum*

INTRODUCTION

Huge quantities of wheat straw are available throughout the World. About 556.35 metric tonnes of wheat strew is estimated to have been produced in the year 2003 all over the world, approximately 65.13 million metric tones have been produced in India alone (FAO , 2004). There is increasing emphasis on the proper management of the straw for which various strategies have been proposed from time to time; a number of these strategies involve its decomposition by microorganisms (Bacon, 1979, Charaya and Mehrotra, 1998). Like many other agricultural wastes, wheat straw also largely consists of lignified cell wall material–cellulose, hemicellulose and lignin being the three primary structural components. Lignin, together with hemicellulose, encrusts the cellulose chains forming a barrier which prevents wetting and access of microbial enzymes (Kirk and Haskin, 1973). Therefore, it would be worthwhile utilizing selected microorganisms for loosening the lignocellulosis bond (Eggins and Seal, 1978). The addition of phosphorus and nitrogen have been reported to enhance the rate of plant litter decomposition (Chekalov, 1955, Gaur *et al.*, 1980 Charaya *et al.*, 1989; Singh *et al.*, 1995; Hadas *et al.*; 1998). The species of *Trichoderma* are known to possess good cellulose decomposing ability (Maheshwari, 2008) Therefore, it would be worthwhile to study the effect of addition of phosphorus and nitrogen containing compounds singly and in combination on the decomposition of straw and its components by *Trichoderma lignorum* isolated from these substrates decomposing naturally in the field in our laboratory.

MATERIALS AND METHODS

To find out the effect of urea and single super phosphate on the ability of the selected fungus to decompose different components of wheat crop residues *in vitro*, the methods followed by Ivarson (1974) and Hering (1967) was used. The wheat internodes, leaves, chaff as well as straw were cut into small pieces and were air-dried. Two g of wheat internodes were placed in 15 conical flasks (250ml capacity); two g of wheat leaves in 15 conical flasks; two g of chaff in 15 conical flasks; and two g straw in 15 conical flasks – thus, total 60 flasks were used. The flask were plugged and autoclaved at 15 lbs/square inch pressure for 30 minutes and then again after 24 hours for 30 minutes. Each set of 15 flasks was treated as under

(i) 10 ml of sterilized water were added to each of a set of three flasks (control);

(ii) 10 ml of sterilised water + inoculum of *Trichoderma lignorum* were added to each of a set of 3 flasks;

(iii) 10 ml of urea solution (0.21%) + inoculum of *Trichoderma lignorum* were added to each of a set of 3 flasks;

(iv) 10 ml single super phosphate solution (1%) + inoculum of *Trichoderma lignorum* were added to each of a set of 3 flasks;

(v) 10 ml of urea solution (0.21%) + single super phosphate solution (1%) + inoculum of *Trichoderma lignorum* were added to each of a set of 3 flasks.

0.21% solution of urea was used because *Trichoderma lignorum* can grow at very low nitrogen level; and such fungi were earlier shown to exhibit significant increase in the rate of decomposition of paddy straw amended with 0.21% urea solution (Charaya, 1985, Jain 1989). 1% single super phosphate amendment is used for cereal straw treatment under Karnal process (Ramachandran, 1989).

At the end of 30 days, the flasks were dried in an oven for 48 hrs at 70°C and the dry weight of the residues were recorded. The loss in the weight of inoculated residues minus the loss in control residues was taken as an index of the capacity of the test fungus to decompose the particular residue.

RESULTS AND DISCUSSION

Results of the study, presented in table 1, reveal that treatment with 0.21% urea or 1% single super phosphate or their combination exhibited highly significant variations with regard to their effect on the decomposition of different components of wheat crop residues by *Trichoderma lignorum in vitro*. Not only this, different substrate exhibited significant differences in their responses to different treatments.

Table 1: Percentage loss (after 30 days) in the dry wt. of unamended wheat crop residues as well as those amended with 0.21% Urea and 1% Single Super phosphate (SSP)—singly and in combination—and inoculated with *Trichoderma lignorum*

Crop Residues	Water + *T. lignorum* (control)	Urea + *T. lignorum*	SSP + *T. lignorum*	SSP + Urea + *T. lignorum*
Internodes	9.85±1.14	14.10±2.16	12.95±0.89	14.55±1.04
Leaves	18.00±2.11	20.70±1.91	18.35±1.43	21.55±3.19
Chaff	17.25±1.16	18.20±2.37	17.40±1.14	19.70±1.97
Mixed	14.70±1.87	17.60±1.26	16.60±1.18	18.50±2.06

Addition of urea had a positive effect on the decomposition of internodes. The same was true when single super phosphate was added to the internode, though the effect of the single super phosphate was less marked than that of urea. The combined effect of urea and single super phosphate was more than that of urea and single super phosphate alone. The addition of urea had strong positive effect as compared to SSP alone in case of leaves. However, in the set of chaff, the addition of SSP had almost negligible positive effect, while urea was slightly more effective. In case of wheat straw, addition of urea had more positive effect as compared to SSP and combined effect appeared to be additive. The rate of decomposition of different components was in the order of leaves > chaff > straw > internodes.

Decomposition of given substrate by a given microorganism depends up to a number of factors. These includes C : N levels (Park, 1976), C : N ratio of internodes is 109.3 while that of leaves is 39.0 (Robinson *et al.*, 1994). Nitrogen is a key nutrient substance for the growth of microorganisms and its sufficient supply is absolutely necessary for the decomposition of the residues. A microbial cells contain 5-15 parts of carbon to one part of nitrogen (Alexander, 1977). The straw contains 0.5% nitrogen and 40% carbon. When it is attacked by decomposers specially fungi, it has only 0.5 units of nitrogen to satisfy the active biological agents which generally require 1.2 to 1.6 units of nitrogen (Alexander, 1977). Thakur and Gupta (2000) as well as Newton (2001) also reported that the application of urea increased microbial decomposition of cellulose.

The addition of phosphorus led to an increase in the rate of decomposition (Stotzky and Norman, 1961 , Zayed *et al.*, 1971). During organic matter decomposition, assimilation of phosphorus into the cytosol of microorganisms takes places (Subba Rao, 1982). The best results were obtained when nitrogen and phosphorus (both) were applied as compared to nitrogen and phosphorus alone (Meena *et al.*, 2002).

Trichoderma lignorum is known to posses good cellulolytic as well as lignolytic activities and good hemicellulolytic ability (Charaya, 2006) *in vitro*. Further analysis of biochemical changes in the crop residues as a result of *in vitro* decomposition in the fungus would reveal the reason behind the differences in the rate of decomposition of different substrate by fungal species.

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IMPACT OF FLYASH WATER ON BIOCHEMICAL CONTENTS OF *ARACHIS HYPOGEA L.*

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ABSTRACT

Arachis hypogea L. plant was grown on ash water released from thermal power plant Deepnagar and the biochemical contents of seed were studied. Free amino acids, Oil, protein content were found decreased at all concentration levels than that of control. Chlorophyll content was seen as fluctuating at some concentration levels .

Key words: Flyash water, Arachis, Biochemical parameters

INTRODUCTION

In India more rapid industrial growth demands more electric power and to fulfill this, new Thermal power stations have been and are being set up. Numerous trace elements present in ash water are considered to be potentially toxic to the plants, animals and human beings (Jones 1978). The consumption of such toxic plant products may cause hazards to human beings. For the detection of harmful and beneficial effects of ash water, on *Arachis hypogea* L. this investigation is undertaken. The paper deals with the effect of fly ash water on growth parameters, oil, free amino acids (FAA), protein, chlorophyll contents of *A. hypogea*. The main purpose of this study is to detect that the ash water is harmful or beneficial to the *A. hypogea*.

MATERIALS AND METHODS

The required ash water for the investigation was obtained from Thermal power plant (TPP) Deepnagar, India. The chemical composition of ash water given in Table 1. *A. hypogea* (Variety G2) was sown and treated with ash water at various concentrations. 10, 20, 40, 60, 80, and 100%. One plot without treatment served as control for the comparison. Amino acid content estimated by the methods of Sadashivam (1990), protein content was estimated by methods of Lowry et al (1951). These estimations were taken under U.V. spectrophotometer. Chlorophyll content was studied by methods of Arnon (1949).

OBSERVATIONS AND RESULTS

Arachis hypogea L. was grown on ash water released from thermal power plant, Deepnagar at various concentrations such as 10, 20, 40, 60, 80 and 100 percent. Free amino acid content was decreased at all the concentrations studied but the more decrement found at 80 and 100 percent concentration level. Oil content decreased at all concentration levels but major inhibition was noted at 80 percent level. Protein content is drastically inhibited at 80% concentration level. Total chlorophyll was seen increased than control at 20% while 60% shows more inhibition. Chlorophyll-a was seen increased at all levels except 10 percent level. Chlorophyll-b mostly inhibited at 60% level but increased at 20 and 40% concentration levels.

This investigations clearly shows that free amino acids, protein content, oil content was adversely affected at all concentration levels, while Chlorophyll-a and Chlorophyll-b increased at 80 and 20 percent concentration levels respectively.

Table 1: Physicochemical analysis of ash water.

Parameters	Values
Temperature 0 C	26
PH	7.8
Free CO2 (mg/l)	38
Alkalinity (mg/l)	29
Chlorides (mg/l)	33
Total hardness (mg/l)	88
Ca hardness (mg/l)	32
Mg hardness (mg/l)	56
Residual chlorine (mg/l)	8.3

Table 2: Biochemical content of *A. hypogea* grown on ash water.

Parameters	Control	10%	20%	40%	60%	80%	100%
Free Amino acids (mg/g)	0.67± 0.16	0.42± 0.05 (-37.31)	0.42± 0.0 (-37.31)	0.45± 0.0 (-32.83)	0.39± 0.17 (-41.79)	0.33± 0.03 (-50.76)	0.30± 0.10 (-55.23)
Oil Content (%)	27.2± 1.19	27.2± 2.15 (+0.0)	26.5± 2.2 (-2.57)	24.2± 0.72 (-11.02)	23.1± 0.12 (-15.07)	23.1± 0.77 (-15.07)	21.0± 0.18 (-22.79)
Protein Contents (mg/g)	36.2± 2.16	30.5± 1.22 (-15.74)	29.0± 1.0 (-21.55)	27.2± 0.0 (-26.48)	24.6± 0.00 (-31.66)	24.7± 0.72 (-33.33)	25.2± 0.84 (-30.95)
Total chl (mg/g)	0.0023	0.0015 (-34.78)	0.0038 (+65.21)	0.0035 (+52.17)	0.0019 (-17.39)	0.0032 (+39.13)	0.0029 (26.08)
Chl- a (mg/g)	0.0014	0.0010 (-28.57)	0.0021 (+50.0)	0.0019 (+35.71)	0.0013 (-7.14)	0.0022 (+57.14)	0.0020 (42.85)
Chl – b (mg/g)	0.009	0.0005 (-44.4)	0.0016 (+77.7)	0.0015 (+67.7)	0.0005 (-44.4)	0.0009 (0.00)	0.0009 (0.00)

±: standard deviation, +: stimulation, -: inhibition, ( ) denotes: - D.F.C.

DISCUSSION

Wong and Wong (1989) reported positive and negative effects of fly ash and fly ash water on germination and seedling growth in Brassica, he also noticed the lower concentration of fly ash water had stimulatory effects and high concentration showed inhibitory effects. (Jones 1978; Petruzelli et al 1987) showed heavy metal uptake by wheat seedling from fly ash water, found no negative effects on the growth of wheat seedling. Karpate and Choudhary (1997) found the damaging effects on cytological parameters of wheat. The inhibitory effects might be because of high level of toxic elements like Boron, arsenic, lead, thorium etc., which are present in ash water. If the plants were irrigated on ash water which may accumulate these elements at high level and such product, if consumed, may have damaging effects on live stock or human being.

CONCLUSION

From the above results it clearly indicate that lower levels of fly ash concentration are beneficial for the yield and biochemical content of B. juncea, while higher concentrations shows damaging effects.

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STUDIES ON *IN VITRO* ANTIMICROBIAL ACTIVITIES OF LEAF EXTRACTS OF *DATURA METEL* LINN.

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ABSTRACT

This study deals with the effect of leaf extract of *Datura metel* Linn. on certain fungi and bacteria. *Aspergillus flavus* has shown 75 per cent growth inhibition at 15 per cent concentration, *Fusarium oxysporum* produced 50 per cent growth inhibition at 5, 10 and 15 per cent concentration. *Enterobacter aerogenes* showed 50 per cent growth inhibition at 5, 10 and 15 per cent concentration, *Escherichia coli* produced 50 per cent growth inhibition at 10 and 15 per cent concentration.

Key words: Antibacterial activity, *Datura metel*, *Growth inhibition*

INTRODUCTION

Plants produce many secondary metabolites and constitute an important source of microbicides and pharmaceutical drugs (Balandrin *et al.*, 1985). Indian systems of medicine have a well documented account of several indigenous medicinal plants and their therapeutic value. Plant produced compounds are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents (Heisey and Gorham, 1992). The secondary metabolites of medicinal plants are being purified, isolated and used in pharmaceutical preparations (Perry, 1980). *Datura metel* Linn. is a common shrubby herb with large purple solitary flowers found in tropical deciduous forests and on waste lands. Indian systems of medicine attach significant medicinal value to this taxon. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et* al., 2005). The present work is to test the antimicrobial activity properties of this herb in *in vitro* condition.

MATERIALS AND METHODS

Leaf samples of *Datura metel* were collected from the herbal garden in the college campus. The fresh leaves (100 gm) were washed thoroughly in sterile water and ground in a blender in 300 ml of sterile water. The extracts were obtained by extracting through vacuum filtration method and added to sterilized PDA (Potato Dextrose Agar) medium for fungi and Muller-Hinton agar medium for bacteria to get different concentrations as given below (Singh and Singh, 1997).

i) 100 ml of medium consisting of 5 ml of extract (5%)
ii) 100 ml of medium consisting of 10 ml extract (10%)
iii) 100 ml of medium consisting of 15 ml of extract (15%)
iv) Only 100 ml of medium as control.

All the sets were sterilized and were poured into sterilized Petri plates in duplicate. Petri plates were inoculated with inoculum of bacteria and fungi. The plates were incubated at 32° ± 1° C for three days in the case of bacteria, and at 28° ± 1° C for five days in the case of fungi (Singh *et al.*, 1994). The growth conditions of bacterial and fungal colony in different concentrations were represented in the form of percentage of growth inhibition and compared with control (without leaf extract).

RESULTS AND DISCUSSION

Two fungi namely *Aspergillus flavus* and *Fusarium oxysporum* and two bacteria namely *Enterobacter aerogenes* and *Escherichia coli* were tested for the antimicrobial activity of the crude leaf extract of *Datura metel*. The results indicate that *Aspergillus flavus* has shown 75% growth inhibition at 15% concentration of leaf extract, whereas 5% and 10% concentrations have shown 50% of growth inhibition. *Fusarium oxysporum* has shown 50% of growth inhibition in all the three concentrations (Table 1).

Table - 1

Inhibitory effects of crude leaf extracts of *Datura metel* Linn.

Organisms	Control	Concentration		
		5%	10%	15%
Aspergillus flavus	++++	++	++	+
Fusarium oxysporum	++++	++	++	++
Enterobacter aerogenes	++++	++	++	++
Escherichia coli	++++	+++	++	++

Note: Control : Medium without leaf extract
++++ : Normal growth
+++ : 25% growth inhibition
++ : 50% growth inhibition
+ : 75% growth inhibition

Enterobacter aerogenes has shown 50% of growth inhibition at all the concentrations, whereas *Escherichia coli* has shown 50% of growth inhibition at 15 and 10% concentration and 25% of growth inhibition at 5% concentration.

The extracts of higher plants are very good source of antibiotics (Fridous *et al*., 1990) against various fungal and bacterial pathogens. Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial drugs. The antimicrobial activities of *Datura metel* leaf extract is evident from its inhibitory effect on growth characters. The difference in the per cent of inhibition varies with different workers used in this study. Similar results were observed in earlier reports of Solanaceae made by Bhattacharjee *et al*. (2005), Cichewicz and Thorpe (1996) and Ramzi *et al.* (2005) with reference to antifungal activity, whereas Svanberg (1992), Hiremath *et al*. (1993) and Dhingra *et al*. (2000) reported similar results with reference to antibacterial activity.

In conclusion, the *in vitro* test on antimicrobial activity of *Datura metel* revealed that the leaf extracts can be used as antimicrobial agents. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

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GENOME WIDE ANALYSIS AND IDENTIFICATION OF GENES RELATED TO CYCLIC NUCLEOTIDE GATED CHANNELS (CNGC) IN *ORYZA SATIVA*

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ABSTRACT

Uptake and translocation of anionic nutrients play essential roles in physiological processes including plant growth, nutrition, signal transduction, and development. Approximately 5% of the *Oryza sativa* genome appears to encode membrane transport proteins. These proteins are classified and several hundred putative transporters have not yet been assigned to families. In this work, we have analyzed the phylogenetic relationships of CNGC (cyclic nucleotide gated channel) which are anion transporters. Phylogenetic trees of the family define the evolutionary relationships of the members to each other. This family contains numerous members, indicating diverse functions in vivo. Closely related isoforms and separate subfamilies exist within the gene family, indicating possible redundancies and specialized functions. To facilitate their further study, which includes alignment of the analyzed genes and their chromosomal locations.

Key words: Cyclic nucleotide gatted channels, Phylogentic analysis, Mapping Anion transporters.

INTRODUCTION

A family of cyclic nucleotide-gated channels (CNGCs), first discovered in barley (Schuurink *et al* 1998), is characterized by the presence at the C-terminus of both cyclic nucleotide and calmodulin binding domains. The 20 members of this family are structurally related to Shaker type K^+ channels with six putative trans-membrane spans and a pore region between spans 5 and 6. A domain in the 4th

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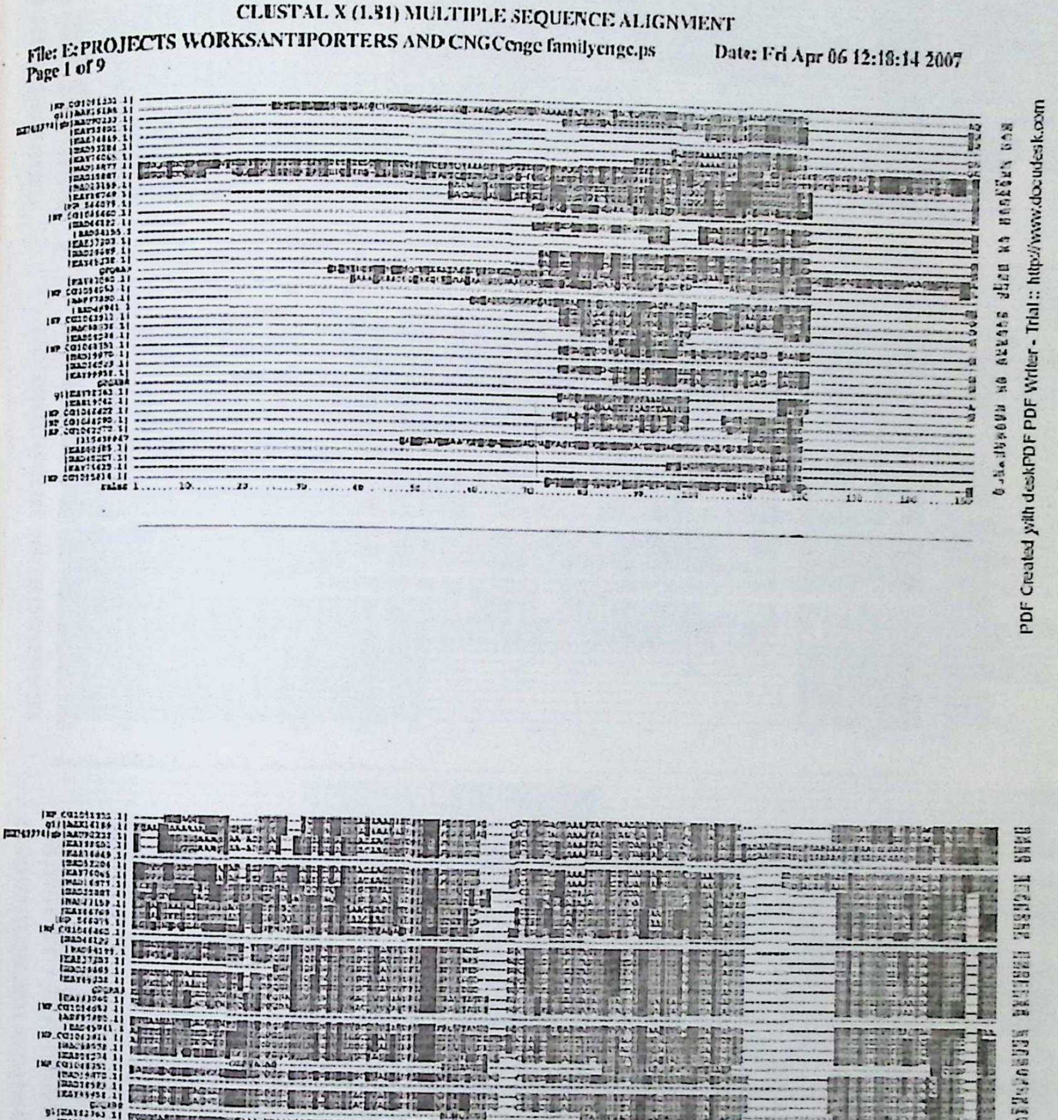
transmembrane span shows similarity to the Shaker type voltage sensor and a C-terminal domain is believed to bind both cyclic nucleotides and calmodulin (Coehler and Neuhaus 2000). Understanding of CNGC function is fragmented. Heterologous expression appears frequently problematic, but the limited data available indicate that gating of plant CNGCs critically depends on the presence of cAMP and/or cGMP (Leng *et al* 1999,2002, Balague *et al* 2003). Recent work further suggests that calmodulin reduced CNGC mediated current, possibly by interfering with cyclic nucleotide binding (Hua *et al* 2003a). Voltage dependence in the form of inward rectification was observed for CNGC^1 and CNGC^2 (Leng *et al* 1999) but not CNGC^4 (47). Functional analysis in heterologous systems further showed that all CNGCs characterized freely conduct K$^+$ and Na$^+$ apart from CNGC^2 which discriminates against Na$^+$ (Leng *et al* 2002, Hua *et al* 2003a), a feature that is unknown in animal CNGCs and shown to be related to an unusual selectivity motif in the pore region (Hua *et al* 2003a) . In plants, the role of CNGCs is less clear. CNGCs have been shown to be present in many species (Talke *et al* 2003). Some evidence suggests a role of CNGCs in heavy metal homeostasis, for example, in tobacco, over expression ofNtCBP4 led to hypersensitivity to Pb^{2+} (Sunkar *et al* 2000). By contrast, expression of a truncated version of NtCBP^4 led to increased tolerance to Pb^{2+} and reduced uptake of this metal ion (Clough *et al* 2000). A detailed characterization of null mutation in AtCNGC^2 and AtCNGC^4 revealed that both mutants exhibit altered responses to pathogen attack. Cngc^2 null mutations lead to a 'defenceno death' phenotype after exposure to pathogens and fail to generate a typical hypersensitivity response or programmed cell death (Chan *et al* 2003) . Cngc^4 mutants show a lesion mimic phenotype where a partial hypersensitive response occurs in the absence of a pathogen. In addition, a loss of function in CNGC^2 affects Ca^{2+} and monovalent cation homeostasis (Li *et al* 2005) whereas CNGC^{10} was recently shown to be involved in plant K$^+$ uptake (Jabs *et al* 1997) . Ca^{2+}, K$^+$ and cyclic nucleotides have all been shown to be involved in early signaling during defence responses (Bindschedler *et al* 2001). For example, the production of reactive oxygen species (ROS) (which have antimicrobial activity) requires cAMP and Ca^{2+} in French bean cells (59) whereas cGMP induces defence gene expression in tobacco suspension. Biochemical studies with a CNGC orthologue from tobacco and CNGC^1 from A. thaliana have elegantly demonstrated that the cyclic nucleotide-binding domain overlaps with that of calmodulin (Arazi *et al* 2000), thereby suggesting that cyclic nucleotides and calmodulin interact in regulation of channel activity. These studies provide some insights into the role of plant CNGCs suggesting their involvement in pathogen responses and possibly heavy metal homeostasis. In addition, functional data show that CNGCs act as monovalent cation transporters in heterologous systems. The latter prompted us to test whether CNGCs might be involved in the uptake and translocation of monovalent cations in planta. Three independent CNGC^3 mutant alleles that showed a consistent phenotype associated with cation stress affected seed germination and distribution of monovalent cations.

MATERIALS AND METHODS

Reference proteins of well-established molecular function, representing each of the protein families investigated, were chosen as query sequences for searches in the rice (*O. sativa*) genome databases. . These reference proteins were CNGC3 (At2g46430), CNGC5 (At5g57940), CNGC^{15} (At2g28260), CNGC^{19} (At3g17690), CNGC^2 (At5g54250). Searches were made using the TBLASTN tool (19) against GenBank database non-redundant (NR), with search specifications for *O. sativa*. The other databases used were Rice Genome Research Program (RGP) (http://rgp.dna.affrc.go.jp/),The Institute of GenomeResearch(TIGR),rice genome annotation database (http://www.tigr.org/tdb/e2k1/osa1/index.shtml) and Universal

Fig.1: Alignment of CNGC family genes



CLUSTAL X (1.81) MULTIPLE SEQUENCE ALIGNMENT

File: E:PROJECTS WORKSANTIPORTERS AND CNGCcngc familycngc.ps Date: Fri Apr 06 12:18:14 2007

Page 1 of 9

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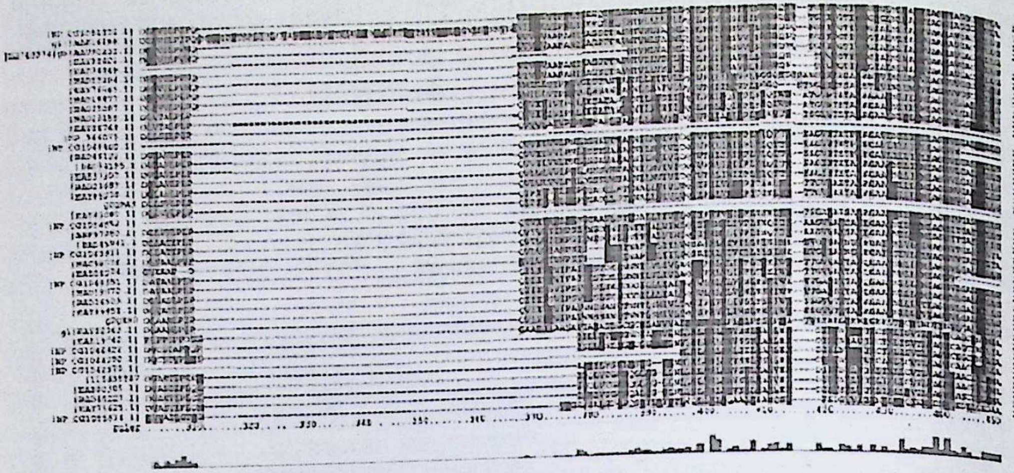
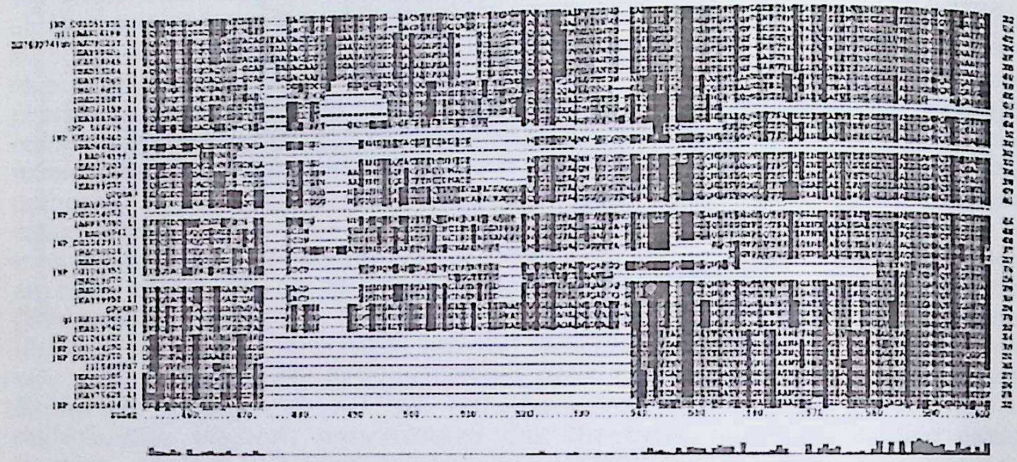
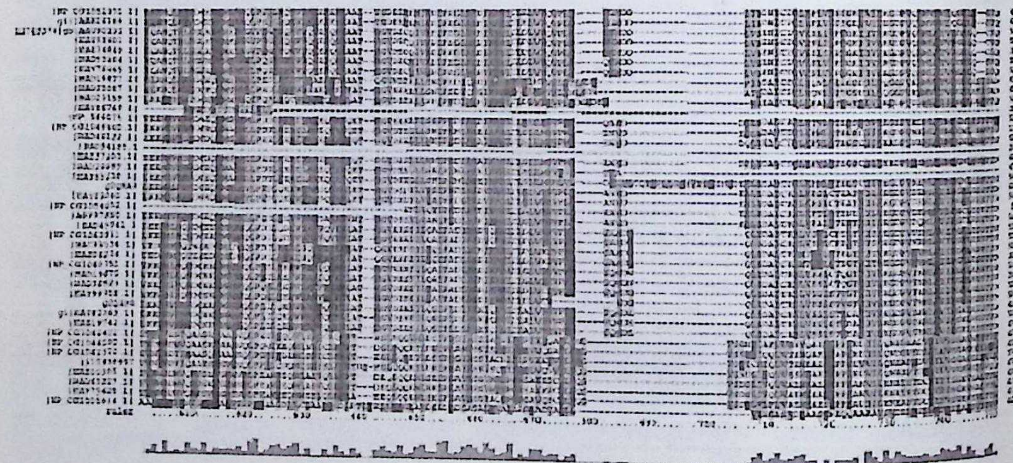
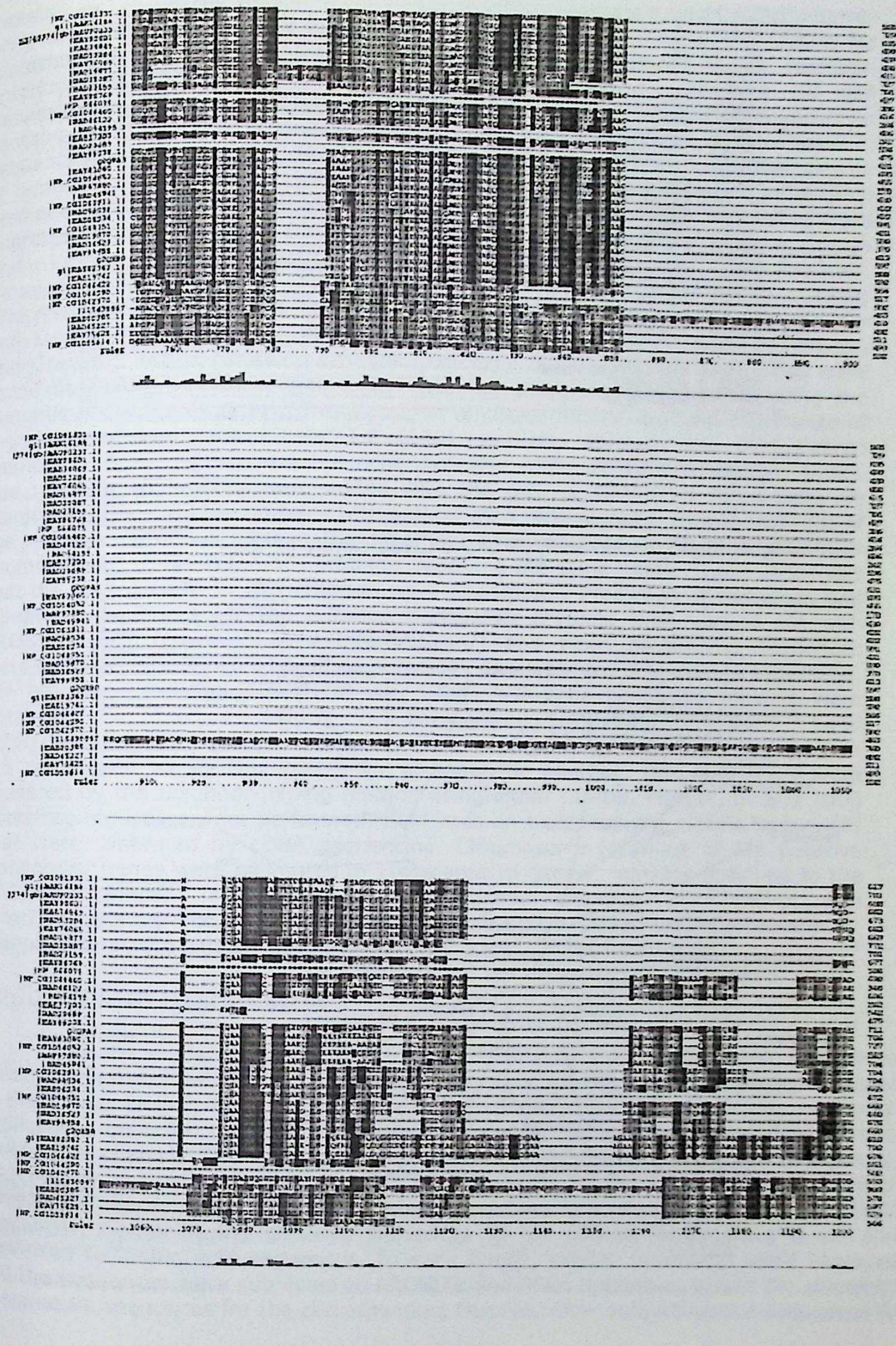








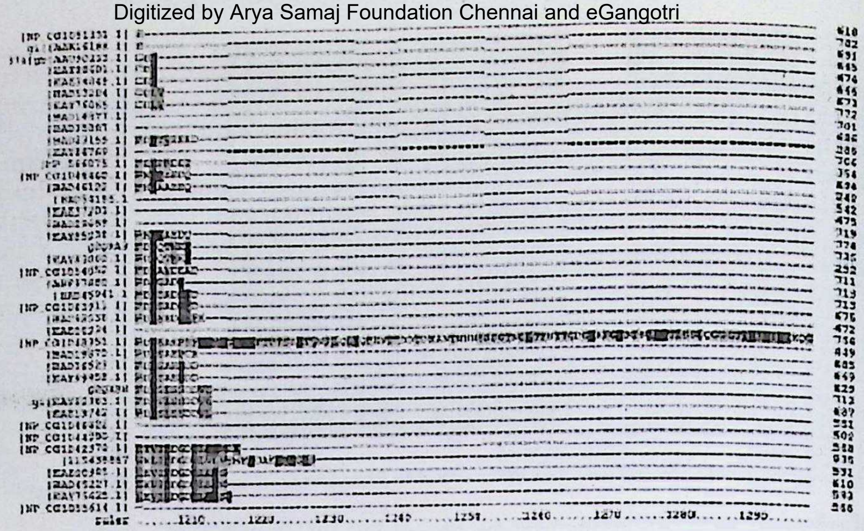
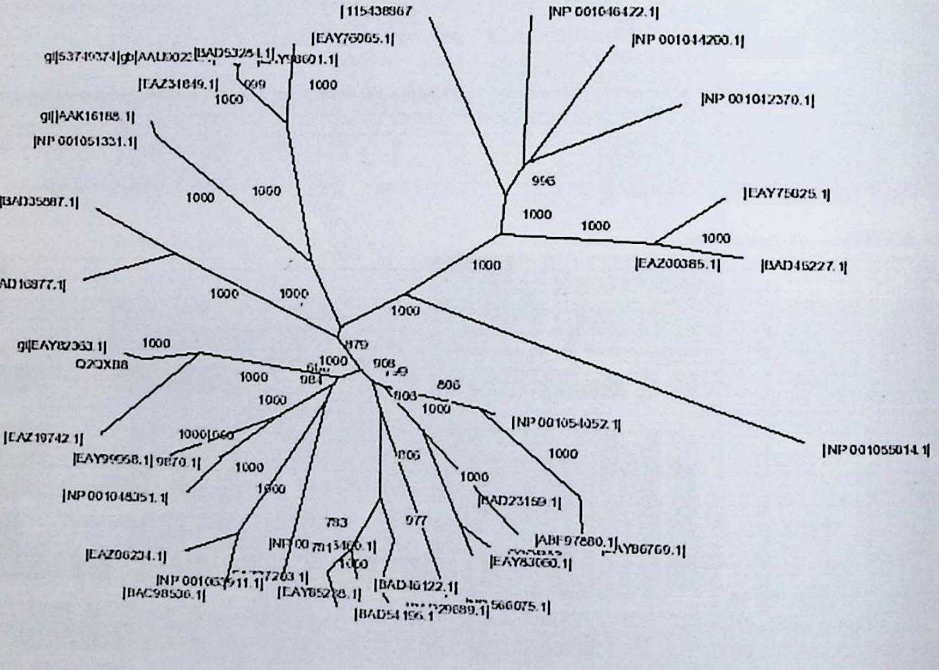


Fig 2: Phylogenetic analysis of the CNGC family genes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Protein resource Uniprot(http://www.ebi.uniprot.org/uniprot-srv/protein/uniProtView). The BLAST server used was that of the National Center for Biotechnology Information (http:// www.ncbi.nlm.nih.gov/BLAST/). As selection criteria of BLAST hits for genomic sequences, a cut off e-value of e- 10 was previously set. The genomic sequences found were used to predict putative genes contained within them. Whenever possible, genes were predicted on the basis of sequences generated by the IRGSP, since these sequences present a higher degree of accuracy. To that end, a mixed procedure was adopted combining ab initio gene prediction algorithms of genomic sequence alignments with similar sequences from expressed genes (ESTs and cDNAs). The prediction algorithms were GenScan (Burge and Karlin, 1997; http:// genes.mit.edu/ GENSCAN.html), GenomeScan; http://genes.mit.edu/ genomescan.html), FGENESH (20); http://www.softberry.Com/berry.phtml? topic=gfind), GeneMark.hmm (Borodovsky and Lukashin, unpublished; http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi) and GrailEXP; http://compbio. ornl.gov/grailexp/). Such expressed sequences were found by BLAST searches against EST and NR databases of GenBank, using the genomic sequence as query. The algorithm of choice for the multiple alignments of protein sequences was ClustalX1.8 [100], available through the BCM Search Launcher server (http://searchlauncher.bcm.tmc.edu/multi-align/multialign. html). The multiple alignments were edited with the help of GENEDOC (Free Software Foundation Inc.). All the proteins with greater than 30% identity, with at least one of the reference proteins used in the searches, were regarded as functionally similar (homologous) to the reference proteins, receiving the same name. Those sequences that did not conform to this criterion were discarded. . Prediction of homology and signature sequences for the putative transporter proteins were carried out with PROSITE (http://www.ebi.ac.uk/InterProScan/) and Pfam databases. Sequences were included into families based on homology and presence of signature sequences. For topology prediction, HMMTOP was used. RGI gene codes for families were obtained from http:// www.gramene.org/Multi/blastview and http://tigrblast.tigr.org/euk-blast/index. Protein alignments obtained with ClustalX 1.8 were used as starting points for phylogenetic analysis. Unrooted trees were prepared by the neighbor-joining method using either Clustal, PHYLIP, or and 1000 bootstrap replicates were performed. Bold lines on trees indicate protein sequences that were confirmed by cDNA sequencing. Chromosome locations of the putative transporter genes were estimated in accordance to genetic markers assigned to the BAC/PAC clones. The site used for genetic distance identification was http://rgp.dna.affrc.go.jp/cgibin/statusdb/stattable.plchr=X&lab=RGP.Chromosomal mapping for all the genes was carried out using MAPINSPECT software.

RESULTS AND DISCUSSION

With the availability of the BGI and the IRGSP data in GenBank, it was possible to construct an overview of CNGC channels in rice. As a starting point, the protein families in CNGC like CNGC^3, CNGC^5, CNGC^{15}, CNGC^{19}, CNGC^2, which have positive molecular implications on anion transport, intracellular targeting and storage in Arabidopsis, were chosen for analysis Taking specific members of these families as query sequences, searches were carried out for orthologous sequences in GenBank, RGP and Uniprot current databases using TBLASTN. After searching the databanks with TBLASTN sequences, clones having genomic sequences to the related family were taken and converted to amino acid sequences. In each family, similar sequences were removed and the sequences were subjected to PROSITE and Pfam databases to see the presence of signature sequences for the corresponding families. After subjecting the sequences to

PROSITE 14 new putative genes, 9 characterized, 2 CNGC families like genes were predicted in CNGC families. Together with five previously reported sequences in CNGC, we analyzed in this study a total of 24 genes related to CNGC channel. The percent of identity for all the sequences was calculated in each family with the corresponding query sequence using GENEDOC. The presence of transmembrane domains was also predicted for all transporter proteins. The result was heterogeneous with majority of proteins presenting 12–15 transmembrane domains, which is close to the 12 observed in Arabidopsis. Phylogenetic analysis of the sequences of transporters revealed that the rice transporters were divergent, showing branches in tree view. The phylogentic analysis shows four branches indicating different transporting function to each family. Some of the orthologous sequences are available as full-length cDNA clones. The expressed sequence tags were mentioned as accession numbers for the sequences. Cyclic nucleotide gated channel family contains a total of forty-two genes and these are named as OsCNGC. These are numbered based on the alignment of the gene with query. The genes, which are showing more than 30% identity, are homologous sequences. The conserved regions were indicated in colors in the following figures. The alignment indicates that the CNGC family genes are well conserved and most of the genes have identical functions.

The phylogenetic analysis showed that the total fourty two genes were grouped into four. This indicates the sub families of the CNGC family. In each group again sub groups were separated based on the branches of the tree diagram.

CONCLUSION

In this work, we have identified and analyzed the genes related to CNGC (cyclic nucleotide gated channel) which are anion transporters. The genes were aligned and used for building of phylogenetic tree. The phylogenetic trees of the family define the evolutionary relationships of the members to each other. After subjecting the sequences to PROSITE 14 new putative genes, 9 characterized, 2 CNGC families like genes were predicted in CNGC families. Together with previously reported sequences in CNGC, we analyzed in this study a total of 42 genes related to CNGC channel. The phylogenetic analysis indicates the isoforms of the genes present in the CNGC family.

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PERFORMANCE OF SOME PROMISING RICE VARIETIES IN LATE PLANTED CONDITIONS

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ABSTRACT

Fifty four promising rice varieties of Eastern India were evaluated for late planting conditions. It is revealed from the study that there is severe infestation of leaf folder and Yellow stem borer in the crop , which resulted the crop loss and decrease in the grain yield. Yellow stemborer damage is highest in varieties like Satabdi (43.3), followed by Jogesh (32.1),Suphala (31.5), Sankar(30.0) showing susceptible reaction and lowest in Bhanja (2.1),Rajeswari(2.4),Jajati (2.2) Jagabandhu(1.0) Shravani(3.1)Uphar (1.1)Mahanadi (2.0)Ramachandi(1.0)Rambha(2.6),Mahalaxmi(2.3)showing resistant reaction. Similarly the leaf folder damage is highest in the varieties Tapaswini(38.5) Lalat(25.0), Geetanjali(14.0) Khandagiri(14.0),Pusa 44(11.0), and lowest in Jagabandhu(4.0).Most of the varieties showed moderate tolerance (5-10% damage). The yield is highest in Pratikhya(4020kg/ha)followed by Pusa 44(4150kg/ha)Samanta (3960) Gouri(3690kg/ha)in irrigated ecology. The yield is highest in Mahalaxmi(5940kg/ha) followed by Rambha(4850kg/ha),Savitri (4800kg/ha) ,Urbasi(4610kg/ha) and lowest in Sankar(990kg/ha), Subhdra (1120kg/ha), Parijat(1320kg/ha),Suphala (1350kg/ha).So the varieties like Mahalaxmi, Rambha,Savitri and Urbasi should be popularized in lowland areas and the varieties like Pratikhya, Pusa -44, Samanta, Go .. should be popularized in irrigated areas in late planted conditions.

Key words : Jogesh, Lalitgiri, Khandagiri, Nilgiri, Sankar, Udaygiri, Keshari, Sidhant, Suphala, Ghanteswari, Badami, Pathara, Rudra, Parijat, Subhadra, Birupa, Shravani, Lalat, Konark, Rajeswari, Bhanja, Sarathi, Samanta, Hema, Kharbela, Daya, Gouri, Jajati, Pratikhya, Gajapati, Pratap, Sebati, Meher, Bhoi, Surendra, Bhuban, Geetanjali, Tapaswini, Padmini, Satabdi, Pusa 44, Jagabandhu, Urbasi, Prachi, Indravati, Manik, Uphar, Mahanadi, Rambha, Jaganath, Mahalaxmi, Kanchan, Ramachandi, Savitri.

INTRODUCTION

Rice is the staple food of India as well as Orissa. It occupies 44.5 Mh in India. In Orissa most of the lands are rainfed lowlands. The yellow stem borer (YSB) is the most predominant pest of rice growing tracts of India.(Islam,1996).Due to climate change, there is severe incidence of pest . Rath and Dani (2010) studied the reaction of pest and yield in rice. Subudhi and Padhi (2008)also studied the YSB reaction over seasons. There is severe flood in the month of July and August which resulted complete damage of the crop. After that, the fields remain fallow in rainfed areas.. In order to overcome this situation, suitable rice varieties should be identified for late planted conditions, so that the farmers of Eastern India will be benefited. Keeping this in view, 54 promising high yielding rice varieties were evaluated for late planted conditions to find out better varieties.

MATERIAL AND METHODS

The experiment was conducted in Central Rice Research Institute, Cuttack. Fifty four promising rice varieties were transplanted in 15th September 2007 to evaluate their performance in late planted conditions. 30 days old seedling were transplanted in Randomized block design with three replications. The recommended agronomic practices were followed. The incidence of insect like yellow stem borer and leaf folder were recorded according to SES Score(IRRI.1996). The grain yield of each varieties were recorded to find out better varieties in late planted conditions.

Table-1 :Yellow stem borer and leaf folder reaction and grain yield in different varieties of Eastern India under late planted condition

	Varieties	ecology	WEH%	reaction	Leaf folder%	reaction	Yield(Kg/ha)
1	Jogesh	U	32.1	HS	4.5	R	1320
2	Lalitgiri	U	19.7	S	7.0	MR	1380
3	Khandagiri	U	20.3	S	14.0	MS	1650
4	Nilgiri	U	11.4	MS	8.0	MR	2340
5	Sankar	U	30.0	HS	7.0	MR	990
6	Udaygiri	U	18.3	S	5.0	R	2040
7	Keshari	U	15.2	MS	5.0	R	2110
8	Sidhant	U	18.1	S	6.0	MR	2200
9	Suphala	U	31.5	HS	6.0	MR	1350
10	Ghanteswari	U	20.7	S	6.0	MR	1900
11	Badami	U	29.4	HS	8.0	MR	1320
12	Pathara	U	16.4	S	8.0	MR	2310
13	Rudra	U	24.3	S	5.0	R	1650
14	Parijat	U	19.1	S	8.0	MR	1320
15	Subhadra	U	26.7	HS	5.0	R	1160
16	Birupa	I	18.9	S	8.0	MR	1650
17	Shravani	I	3.1	R	7.0	MR	2170
18	Lalat	I	11.6	MS	25.0	HS	3300
19	Konark	I	9.2	MR	5.0	R	2310
20	Rajeswari	I	2.4	R	8.0	MR	2970
21	Bhanja	I	2.1	R	12.0	MS	3320
22	Sarathi	I	21.6	S	5.0	R	2310
23	Samanta	I	15.2	MS	12.5	MS	3960
24	Hema	I	5.7	MR	10.0	MR	2100
25	Kharbela	I	13.2	MS	9.0	MR	2640
26	Daya	I	15.2	MS	7.0	MR	2840
27	Gouri	I	9.5	MR	6.0	MR	3690
28	Jajati	I	2.2	R	5.0	R	2680
29	Pratikhya	I	3.9	R	4.5	R	4020
30	Gajapati	I	7.6	MR	8.0	MR	3690
31	Pratap	I	7.9	MR	5.0	R	2970
32	Sebati	I	13.1	MS	5.0	R	2310
33	Meher	I	13.2	MS	8.0	MR	2900
34	Bhoi	I	18.0	S	8.0	MR	3630

35	Surendra	I	4.7	R	8.0	MR	2640
36	Bhuban	I	7.9	MR	7.0	MR	3600
37	Geetanjali	I	16.6	S	14	MS	2450
38	Tapaswini	I	18.7	S	38.5	HS	3400
39	Padmini	I	15.9	S	7.0	MR	2100
40	Satabdi	I	43.3	HS	5.0	R	1500
41	Pusa 44	I	8.3	MR	11.0	MS	4150
42	Jagabandhu	L	1.1	R	4.0	R	2310
43	Urbasi	L	3.7	R	8.0	MR	4610
44	Prachi	L	4.5	R	8.0	MR	1650
45	Indravati	L	5.0	R	5.0	R	2900
46	Manik	L	10.5	MR	6.0	MR	3320
47	Uphar	L	1.3	R	5.0	R	3500
48	Mahanadi	L	2.0	R	5.0	R	3600
49	Rambha	L	2.6	R	5.0	R	4850
50	Jaganath	L	14.8	MS	5.0	R	2850
51	Mahalaxmi	L	2.3	R	5.0	R	5940
52	Kanchan	L	20.7	S	6.0	MR	2650
53	Ramachandi	L	1.0	R	8.0	MR	3700
54	Savitri	L	28.0	HS	10	MR	4800
CV(%)							4.61
CD(5%)							321.1

RESULT AND DISCUSSION

It is revealed from the experiment that the varieties like Shravani,Rajeswari,Bhanja,Ramachandi,Mahalaxmi,Pratikhya,Rambha,Mahanadi,Uphar,Indravati, Prachi,Urbasi,Jagabandhu and Surendra showed resistant reaction to Yellow stemborer.(table-1).The varieties like Jogesh,Sankar,Savitri,Subhadra,Suphala,Satabdi,Badami are highly susceptible. The Satabdi showed highest damage percentage(43.3%). Similarly the leaf folder incidence were also recorded in field conditions. It is observed that Tapaswini showed highest damage i.e 35,8% followed by Lalat (25%),Geetanjali (14%), Khandagiri (14%) and lowest was recorded in Jagabandhu(4.0),Pratikhya(4.5),Jogesh(4.5),Satabdi(5%), Keshari (5%), Jajati(5%). Most of the varieties showed moderately resistant reaction(table-1).Due to incidence of insect, there is severe yield loss. But some varieties did well in such adverse situation. The upland varieties did not perform better. But the irrigated varieties did well in such situation . The yield is highest in Pratikhya(4020kg/ha)followed by Pusa 44(4150kg/ha)Samanta (3960kg/ha) Gouri(3690kg/ha).Most of the lowland varieties performed better than irrigated varieties. The Mahalaxmi yielded highest i.e (5940kg/ha) followed by Rambha (4800kg/ha), Savitri(4800kg/ha),Urbasi(4610kg/ha).Lowest yield was recorded in Prachi (1650kg/ha)and Jagabandhu(2310kg/ha) in lowland conditions.In Irrigated ,lowest yield was recorded in the varieties like Birupa (1650kg/ha),Satabdi (1500kg/ha)and Padmini92100kg/ha).

It is revealed that the varieties Pratikhya, Pusa-44,Gouri and Samanta shoud be cultivated in irrigated conditions where as the varieties like Mahalaxmi,Rambha,Savitri and Urbasi should be popularized and cultivated in lowland areas of Eastern India.

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CONTRIBUTION TO THE CHROMOSOME ATLAS OF THE NEPALESE FLORA II

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ABSTRACT

This research comprises reports of the chromosome counts for 230 taxa of the Nepalese flora within the 7 families viz. Crassulaceae, Cyperaceae, Ranunculaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae and Solanaceae. Present chromosome counts for 19 taxa of Ranunculaceae are also included here. Of these, count of *Clematis montana* is new record for Nepal.

Key words: Nepalese flora, Chromosomal variations, Meiosis, Genetic diversity.

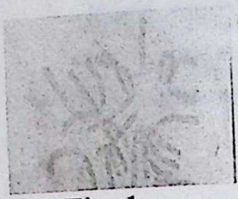
INTRODUCTION

Nepal contains only 1 % of total area of the world (Joshi, 2001) with as many as 7, 000 flowering plants (Shrestha *et al.,* 2000). The genetic diversity of this flora is a valuable heritage for the country.

All together 2, 50,000 of flowering plants are estimated to be known on earth. Of these, only 25 % are cytologically known (Stace, 2000). The Himalayan flora still remains little touched field of investigation (Wakabayashi, 1988; Dhar, 2002). Human intervention has affected global climate change and habitat loss and there is the risk of extinction of about 20-25 % of all of the existing plant diversity in the near future (Kumar and Subramanian, 1986). It is also noteworthy that of the 1223 species, 975 (79.7 %) are endemic or limited to the Himalayas and immediate adjacent areas (Ohba, 1997). This diversity in flora harbors within it an equal genetic diversity, which is yet to be explored to evaluate, maintain and manage its genetic resources.

The chromosome counts report may help to understand chromosomal variations among these taxa other wise the valuable heritage may become extinct before protection. It will also be a valuable document to give recognition of plant genetic heritage resources of the country to scientific world.

Among the taxa within the 7 families reported in this article, viz. Crassulaceae (7 genera), Cyperaceae (21 genera), Ranunculaceae (20 genera), Rubiaceae (39 genera), Saxifragaceae (6 genera), Scrophulariaceae (37 genera)) and Solanaceae (17 genera) that are present in Nepal (Hara and Williams, 1979; Hara *et al.*, 1982; Press *et al.*, 2000; Rajbhandari, 2002-2003), only a few genera from each of these families are cytogenetically known (Table 1).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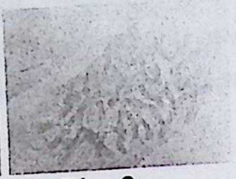
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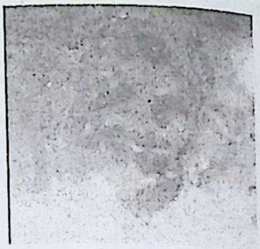
Fig. 3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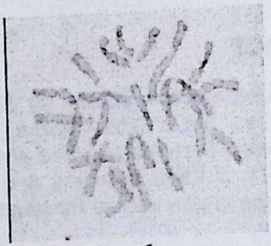
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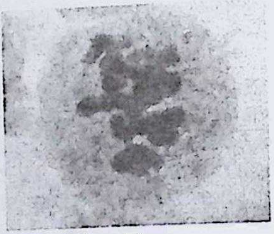
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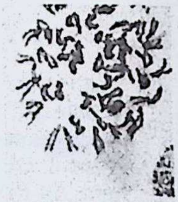
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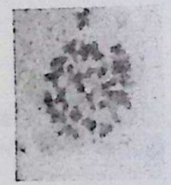
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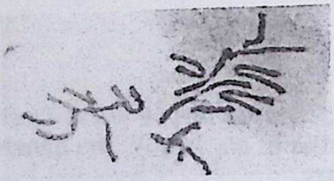
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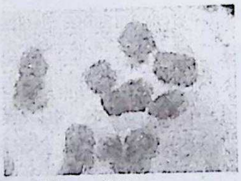
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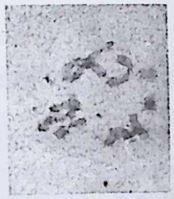
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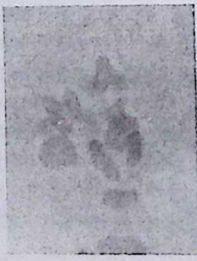
Fig. 13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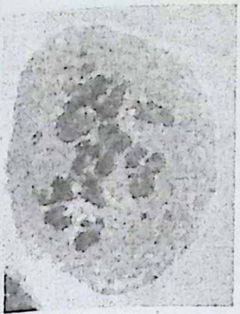
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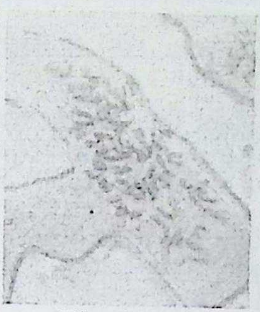
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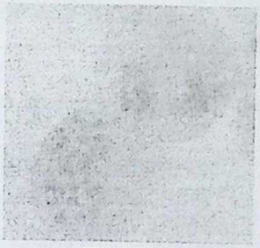
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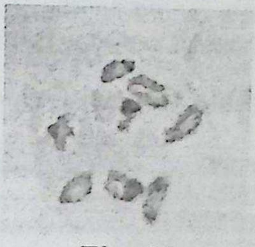
Fig. 24

Fig. 25

Legends for Figures

Fig.1. *Anemone elongata* 2n=14. Fig. 2. *A. rivularis* 2n=32. Fig. 3. *A. rivularis* n=8. Fig. 4. *A. vitifolia* n=8. Fig. 5. *Caltha pelustris* n=15. Fig. 6. *Clematis buchananiana* 2n=16. Fig.7. *C. buchananiana* n=8. Fig. 8. *C. paniculata* 2n=48. Fig. 9. *C. paniculataa* 2n=24. Fig. 10. *C. montana* n=8. Fig.11. *C. smilacifolia* 2n=16. Fig. 12. *C. smilacifolia* n=8. Fig. 13. *Delphinium ajacis* n=8. Fig..14. *D. dictyocarpum* n=8. Fig. 15. *Ranunculus adexifolius* 2n=48. Fig. 16. *R. brotherusi* n=24. Fig. 17. *R. diffusus* n=16. Fig.18. *R. hirtellus* 2n=48. Fig.19. *R. laetus* 2n=16. Fig. 20. *R. laetus* n=8. Fig. 21. *R. sceleratus* 2n=32. Fig. 22. *R. sceleratus* n=16. Fig. 23. *R.* species (hybrid-ornamental) 2n=16. Fig. 24. *R.* species (hybrid-ornamental) n=8. Fig. 25. *Thalictrum foliolosum* 2n=14.

METHODS

The chromosome counts in the present investigation were obtained either from somatic cells of root tips (mitosis) or from microsporogenesis in flower buds (meiosis) according to the methods of Sakya and Joshi (1990) and Cota and Philbrick (1994).

RESULTS

The chromosome counts for different taxa of Ranunculaceae (Fig. 1-25) are reported in the present investigation. Among them, count of *Clematis montana* is a new record for Nepal. The reports for the taxa of present counts are in Table 2 and those of previous counts are listed in Table 3.

Table 1: Previous chromosome counts for the Nepalese genera of different families

Family	Number of Genera	Chromosome counts
Crassulaceae	3	Diploid (Sporophyte)
Cyperaceae	9	Diploid (Sporophyte)
Ranunculaceae	9	Haploid, Diploid (Gametophyte, Sporophyte)
Rubiaceae	8	Haploid, Diploid (Gametophyte, Sporophyte)
Saxifragaceae	4	Haploid, Diploid (Gametophyte, Sporophyte)
Scrophulariaceae	8	Haploid, Diploid (Gametophyte, Sporophyte)
Solanaceae	2	Haploid, Diploid (Gametophyte, Sporophyte)

Table 2: List of voucher number (V. N.) of taxa of the family Ranunculaceae, names of taxa, Place of collection and chromosome number.

V. N.	Taxa	Place of collection (msl)	Chromosome number
114	*Anemone elongata* D. Don	Phulchoki 2600	n=7, 2n=14
115	*A. rivularis* Buch-Ham. ex DC.	Phulchowki, 2480	n=8, 2n=16
116	*A. rivularis* Buch-Ham. ex DC.	Deurali (Way to Gosaithan), 2800	n=16, 2n=32
117	*A. vitifolia* Buch.-Ham. ex DC.	Namobuddha, 1550	n=8, 2n=16
118	*Caltha palustris* Linn.	Kalinchok, 3600	n=15, 2n=30
119	*Clematis buchananiana* DC.	Nagarjun, 1550	n=8, 2n=16
201	*C. montana* Buch.-Ham. Ex DC.	Kalinchowk, 3500	n=8, 2n=16
202	*C. paniculata* Thunb.	Kirtipur, 1300	n=24, 2n=48
203	*C. smilacifolia* Wall.	Kuleswor, 1250	n=8, 2n=16
204	*Delphinium ajacis* L.	Cultivated as ornamental, Kathmandu, 1300	n=8, 2n=16
205	*D. dictyocarpum* DC.	Deurali (Way to Ghorepani), 2980	n=8, 2n=16
206	*D. vestitum* Wall. ex Royle.	Chandanbsri, 3500	n=8, 2n=16
207	*Ranunculus adoxifolius* Hand.-Mazz.	Lauribinayak, 3600	n=24, 2n=48

208	*R. brotherusi* Freyn.	Singompa, 3350	n=24, 2n=48
209	*R. diffusus* DC.	Ghandruk, 2000	n=16, 2n=32
210	*R. hirtellus* Royle ex. D. Don	Laurivinayak, 3600	n=24, 2n=48
211	*R. laetus* Wall.	Kirtipur, 1300	n=8, 2n=16
212	*R. sceleratus* L.	Lalitpur, 1315	n=16, 2n=32
213	*R.* species (hybrid).	Cultvated, Kathmandu, 1250	n=8, 2n=16
214	*Thalictrum foliolosum* DC.	Ghodepani, 2700	n=7, 2n=14

Table 3: Chromosome number reports for the Nepalese flora within the families Crassulaceae, Cyperaceae, Ranunculaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae and Solanaceae.

Taxon/ Family	**Chromosome number**	**Author**	**Distribution/Locality (msl)**
Crassulaceae			
Rhodiola bupleuroides Wall. ex Hook f. & Thomson	2n=20	Wakabayashi (2002)	WCE, 4000
R. bupleuroides Wall. ex Hook f. & Thomson	2n=22	Wakabayashi (2002)	WCE, 3250-4400
R. bupleuroides Wall. ex Hook f. & Thomson	2n=22	Wakabayashi (2002)	Dunche, 1800-2000
R. bupleuroides Wall. ex Hook f. & Thomson	2n=22	Wakabayashi (2002)	Jaljale himal, 3400-3500
R. bupleuroides Wall. ex Hook f. & Thomson	2n=44	Wakabayashi (2002)	Annapurna Himal, 3600-4000
R. bupleuroides Wall. ex Hook. f. & Thomson	2n=66	Wakabayashi (2002)	Jaljale himal, 3900-4200
R. bupleuroides Wall. ex Hook f. & Thomson	2n=88	Wakabayashi (2002)	Annapurna Himal, Marsyangdi Khola, 3000
R. bupleuroides Wall. ex Hook f. & Thomson	2n=110	Wakabayashi (2002)	Annapurna Himal, Marsyangdi Khola, 1800
Sedum filipes Hemsi.	2n=34	Wakabayashi & Ohba (1999)	CE, 2400
S. henrici-roberti Raym (Decne.).-Hamet	2n=48	Wakabayashi & Ohba (1999)	C, 4300-4700
S. magae Raym.-Hamet	2n=28	Wakabayashi & Ohba (1999)	C, 3900-4500
S. multicaule Wall. ex Lindl.	2n=28, 84	Wakabayashi & Ohba (1999)	WCE, 1500-3200
S. oreades Raym.-Hamet	2n=22	Wakabayashi & Ohba (1999)	WCE, 3200-5200
S. triactina Berger	2n=24	Wakabayashi & Ohba (1999)	CE, 2700-3900
S. trullipectalum Hook. & Thompson	2n=70	Wakabayashi & Ohba (1999)	WCE, 3600-4700
Sinocrassula indica (Decne.) A. Berger	2n=22	Wakabayashi & Ohba (1999)	W, 2400
Cyperaceae			
Blysmus compressus (L.) Panz. Ex Link	2n=40	Yano *et al.* (2010)	Lho-Samagaun, 3210

Carex atrofusca Schkuhr subsp. *minor* (Boott) T. Koyama	2n=48	Yano *et al.* (2010)	Samagaun, on way to Manaslu base camp, 4130
C. cruciata Wahlenb.	2n=42	Hoshino (1997)	CE, 1500-3400
C. cruciata Wahlenb.	2n=42	Hoshino *et al.* (2000)	CE, 1500-3400
C. cruenta Nees	2n=44	Hoshino (1997)	WCE, 4000-5600
C. cruenta Nees	2n=44	Hoshino *et al.* (2000)	WCE, 4000-5600
C. duthiei C. B. Clarke	2n=50	Hoshino (1997)	WCE
C. filicina Nees	2n=46	Yano *et al.* (2010)	Bhimthang-Gho, 2990
C. fusiformis Nees	2n=44	Hoshino (1997)	E, 3170-3400
C. fusiformis Nees	2n=44	Hoshino *et al.* (2000)	E, 3170-3400
C. inanis Kunth	2n=42	Yano *et al.* (2010)	Ghap-Namrung, 2130
C. inanis Kunth	2n=42	Yano *et al.* (2010)	Ghap-Namrung, 2390
C. longipes var. *nepalensis* D. Don	2n=44	Hoshino (1997)	CE, 1400-2300
C. longipes var. *nepalensis* D. Don	2n=44	Hoshino *et al.* (2000)	CE, 1400-2300
C. munda Boott	2n=48	Hoshino (1997)	CE, 3200-4100
C. myosurus Nees	2n=44	Hoshino *et al.* (2000)	CE, 800-2900
C. nakaona T. Koyama	2n=45	Hoshino (1997)	CE, 2900-5000
C. remota L.	2n=46	Hoshino (1997)	WCE
C. rufulistolon T. Koyama	2n=46	Yano *et al.* (2010)	Samagaun, on way to Manaslu base camp, 4220
C. speciosa Kunth	2n=44	Yano *et al.* (2010)	Gho-Tal, 2490
C. speciosa Kunth	2n=44	Yano *et al.* (2010)	Ghap-Namrung, 2330
Cyperus haspan L.	2n=26	Yano *et al.* (2010)	Khanchok-Arughat, 960
Eleocharis congesta D. Don	2n=20	Yano *et al.* (2010)	Syange-Bhulbhule, 1130
Eleocharis debilis Kunth	2n=44	Hoshino (1997)	WCE
E. kamtschatica (C. A. Mey) Kom.	2n=44	Hoshino *et al.* (2000)	WCE
E. manillata Lindh. f.	2n=15	Hoshino (1997)	WCE
E. palustris (L.) Roem. & Schult.	2n=16	Hoshino *et al.* (2000)	WC, 2000-3800
E. palustris (L.) Roem. & Schult.	2n=15	Hoshino *et al.* (2000)	WC, 2000-3800

E. tetraquetra Nees ex Wight	2n=20	Hoshino *et al.* (2000)	CE, 1400-3500
Fimbristylis dichotoma (L.) Vahl	2n=10	Hoshino (1997)	WCE
F. dichotoma (L.) Vahl	2n=20	Yano *et al.* (2010)	Gorkha town, 1270
F. litolaris Gaudich.	2n=10	Yano *et al.* (2010)	Khanchok-Arughat, 960
F. schoenoides (Retz.)Vahl	2n=10	Yano *et al.* (2010)	Khanchok-Arughat, 960
Isolepis setacea (L.) R.Br.	n=14	Hoshino *et al.* (2000)	WCE, 2200-3800
I. setacea (L.) R. Br.	2n=32	Yano *et al.* (2010)	Ghap-Namrung, 2240
Kobresia curvata C. B. Clarke	2n=50	Hoshino (1997)	WC,
K. nepalensis (Nees) Kük.	2n= ca.122	Hoshino (1997)	WCE, 2900-5700
K. nepalensis (Nees) Kük.	2n= ca.122	Hoshino *et al.* (2000)	WCE, 2900-5700
Lipocarpha chinensis (Osbeck) Kem	2n=26	Yano *et al.* (2010)	Khanchok-Arughat, 960
Schoenoplectus juncoides (Roxb.) Palla	2n=74	Yano *et al.* (2010)	Khanchok-Arughat, 960
Schoenoplectus juncoides (Roxb.) Palla	2n=74	Yano *et al.* (2010)	Syange- Bhulbhule, 1130
Ranunculaceae			
Aconitum ferox Wall. ex Ser.	2n=20, 34	Joshi & Joshi (2001)	CE, 2100-3800
A. ferox Wall. ex Ser.	n=10, 2n=20	Vaidya (2005)	Daman, 2460
A. ferox Wall. ex Ser.	n=10	Vaidya & Joshi (2010)	Daman, 2460
A. heterophyllum Wall.	2n=16	Joshi & Joshi (2001)	C, 3200-3700
A. laciniatum (Brühl) Stapf.	2n=40	Joshi & Joshi (2001)	CE, 3800-4800
A. laciniatum (Brühl) Stapf.	2n=16	Bhattarai (2001)	Daman, 2400
A. spicatum Stapf.	2n=32	Kurosawa (1966)	E, 2500-4100.
A. spicatum Stapf.	2n=32	Joshi & Joshi (2001)	WCE, 1800-4200
Anemone elongata D. Don	2n=14	Bhattarai (1989)	Serpagaon, 3700
A. elongata D. Don	n=7, 2n=14	Vaidya (2005)	Phulchoki 2600
A. obtusiloba D. Don	2n=14	Vaidya & Joshi (2003)	Cholangpati, way to Gosankunda, 4360
A. obtusiloba D. Don	2n=14	Malla *et al.* (1981)	C, 2700-4000 Dhorpatan
A. obtusiloba D. Don	n=7	Bhattarai (1989)	Chandanbari, 3000
A. obtusiloba D. Don	n=7, 2n=14	Vaidya (2005)	Cholangpati, way to Gosankunda, 4360
A. obtusiloba D. Don	n=7	Vaidya & Joshi (2010)	Kalinchok, 3600

A. obtusiloba subsp. *omalocarpella* Brühl	2n=14	Vaidya & Joshi (2003)	C 2700-4000 Chandanbari, 3000
A. obtusiloba subsp. *omalocarpella* Brühl	n=7, 2n=14	Vaidya (2005)	C, 2700-4000 Chandanbari, 3000
A. rivularis Buch.-Ham. ex DC.	2n=16	Malla *et. al* (1981)	E, 1600-4000 Dhorpatan
A. rivularis Buch.-Ham. ex DC.	n=8, 2n=16	Bhattarai (1989)	E, 1600-4000, Thade
A. rivularis Buch.-Ham. ex DC.	2n=16	Vaidya & Joshi (2004)	Deurali (Way to Gosaithan), 2800
A. rivularis Buch.-Ham. ex DC.	2n= 32	Vaidya & Joshi (2004)	Phulchowki, 2480
A. rivularis Buch.-Ham. ex DC.	n=8, 2n=16	Vaidya (2005)	Deurali (Way to Gosaithan), 2800
A. rivularis Buch.-Ham. ex DC.	n=16, 2n=32	Vaidya (2005)	Phulchowki, 2480
A. rivularis Buch.-Ham. ex DC	n=16, 2n=32	Vaidya & Joshi (2010)	Chandanbari, 3000
A. trullifolia Hook. f. & Thoms.	n=14	Malla *et al.* (1978a)	Topkekhola
A. vitifolia Buch.-Ham. ex DC.	2n=16	Kurosawa (1966)	E, Batasi, 2500.
A. vitifolia Buch.-Ham. ex DC.	n=8, 2n=16	Bhattarai (1989)	WCE, 1300-3300, Chandanbari
A. vitifolia Buch.-Ham. ex DC.	2n=16	Vaidya & Joshi (2003)	Namobuddha, 1550
A. vitifolia Buch.-Ham. ex DC.	n=8, 2n=16	Vaidya (2005)	WCE, 1300-3300, C, Kalinchok, 3400.
A. vitifolia Buch.-Ham. ex DC.	n=8	Vaidya & Joshi (2010)	WCE, 1300-3300, Namobuddha, 1550
Caltha palustris L. var. *himalensis*(D.Don) Mukarjee	2n=32	Kurosawa (1971)	WCE, 2400-4200 C, Kalinchok, 3400.
C. palustris L. var. *himalensis* (D. Don) Mukarjee	2n=32	Joshi & Joshi (2001)	WCE, 2400-4200
C. palustris L.	n=15, 2n=30	Vaidya (2005)	WCE, 2400-4200
Cimicifuga foetida L.	2n=16, 32	Joshi & Joshi (2001)	WCE, 3000-4000
Clematis acuminate DC.	n=8, 2n=16	Vaidya (2005)	Daman, 2300
C. buchananiana DC.	2n=16	Maharjan (1984)	CE, 1800-3500
C. buchananiana DC.	n=8, 2n=16	Vaidya (2005)	Nagarjun, 1550
C. buchananiana DC.	n=8, 2n=16	Vaidya & Joshi (2010)	Nagarjun, 1550
C. connata DC.	n=8, 2n=16	Vaidya (2005)	Kalinchowk, 3200
C. grewiiflora DC.	2n=16	Maharjan (1984)	WCE, 900-1500
C. nepaulensis DC.	2n=16	Joshi & Joshi (2001)	WCE, 1200-2500
C. puberula Hook. f. & Thomson	n=8	Malla *et al.* (1977b)	WCE, 1000-2500 Kalinchok
C. paniculata Thunb.	2n=8	Maharjan (1984)	Kirtipur, 1300

C. smilacifolia Wall.	2n=16	Maharjan (1984)	WC, 300-1800
C. smilacifolia Wall.	n=8, 2n=16	Vaidya (2005)	Kuleshwor 1300
C. smilacifolia Wall.	n=8	Vaidya & Joshi (2010)	Kuleshwor 1250
C. wattii Drummond & Craib.	n=8	Malla *et al.* (1977b)	WCE, 1000-2500 Kelang
Delphinium ajacis L.	2n=16	Pandey (1979)	Cultivated as ornamental, Kathmandu, 1250
D. ajacis L.	n=8, 2n=16	Vaidya (2005)	Cultivated as ornamental, Lalitpur 1250
D. ajacis L.	n=8	Vaidya & Joshi (2010)	Cultivated as ornamental, Lalitpur 1250
D. altissimum Wall.	n=8	Malla *et al.* (1977b)	WCE, 1000-2500 Godawari. 1375
D. cashmerianum Royle	2n=16	Malla *et al.* (1981)	C. Nepal 4100-4200, Godawari, 1375
D. dictyocarpum DC.	n=8, 2n=16	Vaidya (2005)	Deurali (Way to Ghorepani), 2980
D. dictyocarpum DC.	n=8	Vaidya & Joshi (2010)	Deurali (Way to Ghorepani), 2980
D. scabriflorum D. Don	2n=16	Malla *et al.* (1984)	WE, 1500-2500, Royal Botanical Garden, 1275.
D. vestitum Wall. ex Royle	n=8	Malla *et al.* (1978b)	Chandanbari, Gosainkunda, 4200
D. vestitum Wall. ex Royle	n=8, 2n=16	Vaidya (2005)	WCE, 2700-4700
D. vestitum Wall. ex Royle	n=8	Vaidya & Joshi (2010)	WCE, 2700-4700, Ghorepani, 2750
Oxygraphis polypetala (Royle) Hook. f. & Thoms.	n=8, 2n=16	Vaidya (2005)	Ghorepani, 2750
Ranunculus adoxifolius Hand.-Mazz.	2n=48	Kurosawa (1971)	E, 1600-4300
R. adoxifolius Hand.-Mazz.	n=24, 2n=48	Vaidya (2005)	Laurivinayak, 3600
R. brotherusi Freyn.	n=24, 2n=48	Vaidya (2005)	Singompa,3350
R. brotherusi Freyn. var. *brotherusi*	2n=32	Kurosawa (1971)	E, 3000-5000
R. diffusus DC.	2n=16	Pandey (1979)	WC, 1600-2000
R. diffusus DC.	n=8	Singh & Sakya (1980)	Chandragiri, 1850
R. diffusus DC.	2n=32	Vaidya & Joshi (2003)	Rasuwa district, 2050
R. diffusus DC.	n=16, 2n=32	Vaidya (2005)	Ghandruk, 2000
R. diffusus DC.	n=16	Vaidya & Joshi (2010)	Rasuwa, 2050
R. hirtellus Royle ex D. Don	n=16,24, 2n=32, 48	Vaidya (2005)	Laurivinayak, 3600
R. laetus Wall.	2n=16	Vaidya & Joshi (2003)	Kirtipur,1300 ; Panighat, 1620
R. laetus Wall.	n=8, 2n=16	Vaidya (2005)	EC, 1300-4400

R. laetus Wall.	n=8, 2n=16	Vaidya & Joshi (2008)	Kirtipur, 1300 ; Namobuddha ,1540
R. laetus Wall.	n=8	Vaidya & Joshi (2010)	Kirtipur, 1300
R. pulchellus C.A. Meyer	n=24, 2n=48	Vaidya (2005)	Chandanbari, 3360
R. sceleratus L.	2n=32	Vaidya & Joshi (2003)	Lalitpur district, 1315
R. sceleratus L.	n=16, 2n=32	Vaidya (2005)	Lalitpur district, 1315
R. sceleratus L.	n=16	Vaidya & Joshi (2010)	Lalitpur district, 1313
Thalictrum chelidonii DC.	n=7	Malla *et al.* (1977a)	WCE, 2300-3500, Ghopte
T. cultratum Wall.	n=7	Malla *et al.* (1979)	WCE, 2400-4200
T. cultratum Wall.	n=7, 2n=14	Vaidya (2005)	Phulchowki, 1800
T. cultratum Wall.	n=7	Vaidya & Joshi (2010)	Phulchowki, 1800
T. foliolosum DC.	n=7, 2n=14	Vaidya (2005)	Ghodepani, 2700
T. foliolosum DC.	n=7	Vaidya & Joshi (2010)	Ghandruk, 2010
T. javanicum Blume	n=14, 2n=28	Vaidya (2005)	Phulchowki, 1800
T. rotundifolium DC.	n=7	Malla *et al.* (1977b)	CE, 1200-2700,
Rubiaceae			
Kohautia gracilis (Wall.) DC.	2n=36	Malla *et al.* (1984)	Mahendra Nagar, 72
Luculia gratissima (Wall.) Sweet	n=22	Malla *et al.* (1974)	Royal Botanical garden, Godawari, 1275
Mussaenda macrophylla Wall.	n=11	Malla *et al.* (1974)	C, 1300, Bhutbhule
Mussaenda macrophylla Wall.	n=11	Singh and Sakya (1980)	Chandragiri hill, 1690
Neanotis gracilis (Hook. f.) W. H. Lewis	2n=18	Malla *et al.* (1978a)	Godawari, 1300
Rubia sikkimensis Kurz	2n=ca. 132	Kurosawa (1966)	Below Yektin, E. Nepal, 1400
Spermadictyon suaveolens Roxb.	n=10	Malla *et al.* (1977b)	Godawari, 1300
Wendlandia puberula DC.	n=11	Malla *et al.* (1978a)	Godawari Botanical Garden, 1275
Wendlandia coriacea (Wall.) DC.	2n=22	Oginuma *et al.* (1994)	Godawari Botanical Garden, 1275
Xeromorphis spinosa (Thunb.) Keay	2n=22	Joshi and Joshi (2001)	WCE, 100-1200
Saxifragaceae			
Chrysosplemium lanuginosum Hook. f. & Thoms.	n=7, 2n=14	Kurosawa (1966)	Bhuspate Danra, E, 2900
C. nepalense D. Don	2n=24	Kurosawa (1966)	Bandukay Bhanjyang, E, 3100.

Hydrangia anomala D. Don	n=12	Malla *et al.* (1977a)	Sungure
Pamassia nubicola Wall.	n=9	Malla *et al.* (1979)	-
P. pusilla Wall.	n=9	Malla *et al* (1979)	-
P. wightiana Wall.	2n=36	Malla *et al.* (1981)	Dhorpatan,
Saxifraga aristulata Hook. f. & Thoms.	2n=16	Wakabayashi & Ohba (1997a)	WCE, 4200-5600
S. brunonis Wall. ex Ser.	2n=16	Wakabayashi & Ohba (1997a)	WCE, 2400-5600
S. cordigera Hook. f. & Thoms.	2n=16	Wakabayashi & Ohba (1997a)	E, 4100-5100
S. diversifolia Wall. ex Ser.	2n=20	Malla *et al.* (1984)	Chandanbari, 4200
S. diversifolia Wall. ex ser.	2n=16	Wakabayashi & Ohba (1997a)	WCE, 2400-4800
S. gageana W. W. Smith	2n=22	Wakabayashi & Ohba (1988)	Rolwaling himal area, 4100, Shorong Himal area, 4050-4350
S. gageana W. W. Smith	2n=22, 33	Wakabayashi & Ohba (1997a)	Rolwaling himal area, 4100, Shorong Himal area, 4050-4350
S. gageana W. W. Smith	2n=22	Wakabayashi (2002)	Rolwaling, 4100; Shorong Himal area, 4051-4550; Hinko valley, 3800-4050
S. gageana W. W. Smith	2n=33	Wakabayashi (2002)	Shorong himal area, 4000
S. harai H. Ohba & Wakeb.	2n=48	Wakabayashi & Ohba (1997a)	E, 4450-4700
S. hookeri Engl. & Irmsch.	2n=16	Wakabayashi & Ohba (1997a)	CE, 3000-4500
S. mallae H. Ohba & Wakeb.	2n=16	Wakabayashi & Ohba (1997a)	CE, 3979-5000
S. melanocentra Franch.	2n=88, 110	Wakabayashi & Ohba (1997a)	CE, 4100-4900
S. melanocentra Franch.	2n=88	Wakabayashi (2002)	Jaljale himal area, 4400
S. melanocentra Franch.	2n=110	Wakabayashi (2002)	Jaljale himal area, 4500
S. montanella H. Sm.	2n=16	Wakabayashi & Ohba (1997a)	E, 4100-4600

S. pallida Wall. ex Ser.	2n=22	Wakabayashi & Ohba (1997a)	WCE, 3200-4800
S. pallida Wall ex Ser.	2n=22	Wakabayashi (2002)	Shorong himal area, 3200-3350, Jaljale himal area, 3500-3900, Hinku valley area, 3350-4000
S. parnassifolia D. Don	2n=16	Wakabayashi & Ohba (1997a)	WCE, 1900-4900
S. pseudo-pallida Engl. & Irmsch.	2n=66	Wakabayashi & Ohba (1997a)	WCE, 3800-5600
S. pseudo-pallida Engl. & Irmsch.	2n=66	Wakabayashi (2002)	Rolwaling himal area, 4800, Shorong himal area, 4300-4350, Jaljale himal area, 3650-4500, Hinku valley area, 4100-4720
S. saginoides Hook. f. & Thompson	2n=32	Wakabayashi & Ohba (1997a)	WCE, 4200-5200
S. sikkimensis Engl.	2n=16, 24	Wakabayashi & Ohba (1997a)	CE, 3600-5200
S. sphaeradena H. Sm.	2n=16	Wakabayashi & Ohba (1997a)	Alpine Zone
Scrophulariaceae			
Antirrhinum majus L.	2n=16	Rajbansi (1982)	Cultivated, Kathmandu, 1250
A. speciosum Gray	2n=16	Rajbansi (1982)	C, 1300
Ellisiophyllum pinnatum (Wall.) Makino	2n=18	Kurosawa (1966)	Bhuspate Danra, E. Nepal, 2900.
Linaria bipartita (Vent.) Willd.	2n=12	Rajbansi (1982)	C, 1300
Lindenbergia grandiflora (Buch.-Ham.)Benth.	n=15	Malla *et al.* (1977b)	Godawari, 1300
Pedicularis anserantha T. Yamaz var. *elevatogaleata* T. Yamaz.	2n=28, 28+1B	Amano (1997)	WC, 3900-4000, Annapurna Himal
P. anserantha T. Yamaz.var. *elevatogaleata* T. Yamaz	2n=28, 29 (28+1B)	Amano (1999)	WC, 3900-4000, Mustang district
P. clarkei Hook. f.	n=8	Malla *et al.* (1977b)	CE, 3600-4200
P. confertiflora Prain	2n=16	Amano (1997)	WCE, 4100-4900, Annapurna Himal
P. confertiflora Prain	2n=16	Amano (1999)	WCE, 4100-4900, Mustang district
P. confertiflora Prain	2n=16	Amano (2002)	WCE, 4100-4900, Alpine and sub-alpine regions
P. furfuracea Wall. ex Benth.	n=8	Malla *et al.* (1977c)	CE, 2800-4100, Chandanbari
P. gracilis Wall. ex Benth.	2n=16	Amano (1997)	WCE, 2200-3800, Annapurna Himal area

P. gracilis Wall. ex Benth.	2n=16	Amano (1999)	WCE, 2200-3800, Mustang district
P. hoffineisteri Klotzsch	2n=16, 32	Amano (1997)	WCE, 2300-3900, Annapurna Himal area
P. hoffineisteri Klotzsch	2n=16, 32	Amano (1999)	WCE, 2300-3900, Mustang district
P. longiflora ssp. *tubiformis* (Klorzsch) Pennell	2n=14	Amano (1997)	WCE, 3300-5000, Annapurna Himal area
P. longiflora ssp. *tubiformis* (Klorzsch) Pennell	2n=14	Amano (1999)	WCE, 3300-5000, Mustang district
P. longiflora ssp. *tubiformis* (Klorzsch) Pennell	2n=14	Amano (2002)	WCE, 3300-5000, Alpine and sub-alpine regions
P. megalantha D. Don	n=8	Malla *et al.* (1979)	CE, 2800-4300, Phokte-Mandada
P. mollis Wall.	n=8	Malla *et al.* (1977b)	WCE, 2600-4500, Rolwaling Valley
P. nodosa Pennell	2n=16	Amano (1997)	C, 3400-4300, Annapurna Himal area
P. nodosa Pennell	2n=16	Amano (1999)	C, 3400-4300, Mustang district
P. nodosa Pennell	2n=16	Amano (2002)	C, 3400-4300, Alpine and sub-alpine regions
P. pauciflora (Prain) Pennell	2n=16, 32	Amano (1997)	E, 3600-4600, Annapurna Himal area
P. pauciflora (Prain) Pennell	2n=16, 32	Amano (1999)	E, 3600-4600, Mustang district
P. pauciflora (Prain) Pennell	2n=16	Amano (2002)	E, 3600-4600, Alpine and sub-alpine regions
P. scullyana Prain ex Maxim.	2n=16	Amano (1997)	WCE, 3700-5000, Annapurna Himal area
P. scullyana Prain ex Maxim.	2n=16	Amano (1999)	WCE, 3700-5000, Mustang district
Torenia fournieri Linden & Fourn.	2n=18	Rajbansi (1982)	C, 1200
Veronica robusta (Prain) Yamazaki	n=21	Malla *et ai.* (1977a)	CE, 2300-4700. Phulchoki
Wulfenia nepalensis Yamazaki	2n=16	Kurosawa (1971)	Phulchoki, 2600
Solanaceae			
Cestrum fasciculatum Miers	n=8	Singh and Sakya (1980)	Chandragiri hill, 1650
C. nocturnum L.	n=8	Singh and Sakya (1980)	Chandragiri hill, 1600
C. parqui L'Her.	n=8	Singh and Sakya (1980)	Chandragiri hill, 1485
Solanum aculeatissimum Jacq.	n=12	Singh and Sakya (1980)	Chandragiri hill, 1425
S. anguivi Lam.	n=12, 2n=24	Shrestha ((1987)	WCE, 250-2300
S. anguivi Lam.	n=12, 2n=24	Shrestha & Joshi (1992)	WCE, 250-2300
S. erianthum D. Don	n=12, 2n=24	Shrestha ((1987)	WCE, 200-1400

S. erianthum D. Don	n=12, 2n=24	Shrestha & Joshi (1992)	WCE, 200-1400
S. indicum L.	n=12	Singh and Sakya (1980)	Chandragiri hill, 1470
S. jasminoides Paxton	n=12, 2n=24	Shrestha ((1987)	C, Cultivated, Kirtipur, 1300
S. jasminoides Paxton	n=12, 2n=24	Shrestha & Joshi (1992)	Cujltivated, 1300
S. khasianum Clarke	n=12, 2n=24	Shrestha ((1987)	C, 1300
S. khasianum Clarke	n=12, 2n=24	Shrestha & Joshi (1992)	C, 1300
S. nigrum L.	n=36	Singh and Sakya (1980)	Chandragiri hill, 1590
S. nigrum L.	n=12, 2n=24	Shrestha ((1987)	Kathmandu, 1300
S. nigrum L.	n=12, 2n=24	Shrestha & Joshi (1992)	WCE, 900-2900
S. pseudocapsicum L.	n=12	Singh and Sakya (1980)	Chandragiri hill, 1590
S. pseudocapsicum L.	n=12, 2n=24	Shrestha ((1987)	CE, 1400-1500
S. pseudocapsicum L.	n=12, 2n=24	Shrestha & Joshi (1992)	CE, 1400-1500

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